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A GENE CODING FOR A PROTEIN REGULATING AUREOBASIDIN SENSITIVITY

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(57) Claim

[Claim 1] An isolated gene coding for a protein which regulates aureobasidin sensitivity.

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Invention Title: A GENE CODING FOR A PROTEIN REGULATING AUREOBASIDIN SENSITIVITY

The following statement is a full description of this invention, including the best method of performing it known to us:

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[Designation of Document] Specification

[Title of the Invention] A GENE CODING FOR A PROTEIN REGULATING AUREOBASIDIN SENSITIVITY

[Detailed Description of the Invention]

[Field of Industrial Application]

This invention relates to a protein regulating the sensitivity to an antimycotic aureobasidin and a gene coding for this protein, namely, a gene coding for a protein regulating aureobasidin sensitivity. The present invention further relates to a series of the uses of the protein and the gene. Furthermore, it relates to an antibody against this protein and the use of the same.

[Prior Art]

Systemic mycoses including candidiasis have increased with an increase in immunocompromised patients in recent years due to, for example, the extended use of immunosuppressive drugs and acquired immunodeficiency syndrome (AIDS), and as opportunistic infection due to microbial substitution caused by the frequent use of widespectrum antibacterial antibiotics. Although drugs for treating mycoses such as amphotericin B, flucytosine and azole drugs (for example, fluconazole and miconazole) are now used to cope with this situation, none of them can achieve a satisfactory effect. Also, known diagnostic drugs are insufficient. For candidiasis, in particular, although there have been known several diagnostic drugs (for

example, CAND-TEC for detection of candida antigen and LABOFIT for detection of D-arabinitol),none of them gives any satisfactory results in specificity or sensitivity.

The reasons for the delay in the development of remedies and diagnostic drugs for mycoses as described above are that fungi causing the mycoses are eukaryotic organisms similar to the host (i.e., man) and thus not largely different from man and that knowledges of fungi, in particular, pathogenic fungi are insufficient. Therefore it is difficult to distinguish fungi from man or to selectively kill fungi, which is responsible for the delay in the development of drugs for mycoses.

Recently the application of genetic engineering techniques such as antisense or PCR to the treatment and diagnosis of mycoses has been expected. However known genes which are applicable thereto and/or proteins coded for by these genes are rare (PCT Pamphlet WO92/03455). Regarding pathogenic fungi, there have been cloned in recent years an acid protease gene, which has been assumed to participate in the pathogenicity of *Candida albicans* (hereinafter referred to simply as *C. albicans*) and *Candida tropicalis* (hereinafter referred to as *C. tropicalis*) causing candidiasis [B. Hube et al., J. Med. Vet. Mycol., 29, 129 - 132 (1991); Japanese Patent Laid-Open No. 49476/1993; and G. Togni et al., FEBS Letters, 286, 181 - 185 (1991)], a calmodulin gene of

C. albicans [S.M. Saporito et al., Gene, 106, 43 - 49 (1991)] and a glycolytic pathway enzyme enolase gene of C. albicans [P. Sundstrom et al., J. Bacteriology, 174, 6789 - 6799 (1991)]. However, each of these genes and proteins coded for thereby is either indistinguishable from nonpathogenic fungi and eukaryotic organisms other than fungi or, if distinguishable therefrom, cannot serve as a definite action point for exhibiting any selective toxicity.

Aureobasidin [Japanese Patent Laid-Open No. 138296/1990, No. 22995/1991, No. 220199/1991 and No. 279384/1993, Japanese Patent Application No. 303177/1992, J. Antibiotics, 44 (9), 919 - 924, ibid., 44 (9), 925 - 933, ibid., 44 (11), 1187 - 1198 (1991)] is a cyclic depsipeptide obtained as a fermentation product of a strain Aureobasidium pullulans No. R106. It is completely different in structure from other antimycotics. As Tables 1 and 2 show, aureobasidin A, which is a typical aureobasidin compound, exerts a potent antimycotic activity on various yeasts of the genus Candida including C. albicans which is a pathogenic fungus, Cryptococcus neoformans, Histoplasma capsulatum, Blastomyces dermatitidis and fungi of the genus Aspergillus (Japanese Patent Laid-Open No. 138296/1990) but has an extremely low toxicity in mammal. Thus this compound is expected to be useful as an antimycotic being excellent in selective toxicity.

Hereinafter, Candida, Cryptococcus and Aspergillus will be abbreviated respectively as C., Cr. and A.

[Table 1]

Test strain	TIMM No.	MIC (μg/ml)
C. albicans	0136	≦0.04
C. albicans var. stellatoidea	1308	≦0.04
C. tropicalis	0312	0.08
C. kefyr	0298	0.16
C. parapsilosis	0287	0.16
C. krusei	0270	≦0.04
C. guilliermondii	0257	0.08
C. glabrata	1062	≦0.04
Cr. neoformans	0354	0.63
Cr. terreus	0424	0.31
Rhodotorula rubra	0923	0.63
A. fumigatus	0063	20
A. clavatus	0056	0.16

[Table 2]

Test strain	TIMM No.	MIC(μg/ml)
A. nidulans	0112	0.16
A. terreus	0120	5
Penicillium commune	1331	1.25
Trichophyton mentagrophytes	1189	10
Epidermophyton floccosum	0431	2.5
Fonsecaea pedrosoi	0482	0.31
Exophiala werneckii	1334	1.25
Cladosporium bantianum	0343	0.63
Histoplasma capsulatum	0713	0.16
Paracoccidioides brasiliensis	0880	0.31
Geotrichum candidum	0694	0.63
Blastomyces dermatitidis	0126	0.31

[Problems to be Solved by the Invention]

Each of the conventional antimycotics with a weak toxicity shows only a fungistatic effect, which has been regarded as a clinical problem. In contrast, aureobasidin has a fungicidal effect. From this point of view, it has been urgently required to clarify the mechanism of the selective toxicity to fungi of aureobasidin. However this mechanism still remains unknown.

Under these circumstances, the present invention aims at finding a novel protein regulating aureobasidin sensitivity through the clarification of the mechanism of the selective toxicity to fungi of

aureobasidin. Accordingly, the present invention aims at finding a gene coding for a protein regulating aureobasidin sensitivity, providing a process for cloning this gene and the protein regulating aureobasidin sensitivity which is encoded by this gene, further providing an antisense DNA and an antisense RNA of this gene, providing a nucleic acid probe being hybridizable with this gene, providing a process for detecting this gene with the use of the nucleic acid probe, providing a process for producing the protein regulating aureobasidin sensitivity by using this gene and providing an antibody against the protein regulating aureobasidin sensitivity, and a process for detecting the protein regulating aureobasidin sensitivity by using this antibody.

[Means for Solving the Problems]

The present invention may be summarized as follows. Namely, the first invention of the present invention relates to an isolated gene coding for a protein regulating aureobasidin sensitivity, that is, a gene regulating aureobasidin sensitivity. The second invention relates to a process for cloning a gene regulating aureobasidin sensitivity which is characterized by using the gene regulating aureobasidin sensitivity of the first invention or a part thereof as a probe. The third invention relates to a nucleic acid probe which is hybridizable with a gene regulating aureobasidin sensitivity and comprises a sequence consisting of 15 or more bases. The fourth

invention relates to an antisense DNA of a gene regulating aureobasidin sensitivity. The fifth invention relates to an antisense RNA of a gene regulating aureobasidin sensitivity. The sixth invention relates to a recombinant plasmid having a gene regulating aureobasidin sensitivity contained therein. The seventh invention relates to a transformant having the above-mentioned plasmid introduced thereinto. The eighth invention relates to a process for producing a protein regulating aureobasidin sensitivity by using the above-mentioned transformant. The ninth invention relates to an isolated protein regulating aureobasidin sensitivity. The tenth invention relates to an antibody against a protein regulating aureobasidin sensitivity. The eleventh invention relates to a process for detecting a protein regulating aureobasidin sensitivity by using the above-mentioned antibody. The twelfth invention relates to a process for detecting a gene regulating aureobasidin sensitivity by the hybridization which is characterized by using the nucleic acid probe of the third invention of the present invention. The thirteenth invention relates to a process for screening an antimycotic by using the above-mentioned transformant or a protein regulating aureobasidin sensitivity.

The present inventors have found out that fungi such as Schizosaccharomyces pombe (hereinafter referred to simply as Schizo. pombe) and Saccharomyces

cerevisiae (hereinafter referred to simply as S. cerevisiae) and, further, mammalian cells such as mouse lymphoma EL-4 cells are sensitive to aureobasidin, as Table 3 shows.

[Table 3]

Test strain or cell	MIC(μg/ml)
Schizo. pombe	0.08
S. cerevisiae	0.31
mouse lymphoma EL-4	10
mouse lymphoma L5178Y	100
NRK-49F	12.5

The present inventors have mutagenized a wild-type strain of Schizo. pombe or S. cerevisiae, sensitive to aureobasidin, to thereby give resistant mutants. We have further successfully isolated a gene capable of confering aureobasidin resistance (a resistant gene) from these resistant mutants and another gene capable of imparting aureobasidin sensitivity (a sensitive gene) from the corresponding sensitive cells. Furthermore, We have disclosed the existence of a protein encoded by each of these genes. By culturing cells which have been transformed by introducing the above-mentioned gene, We have succeeded in the expression of this gene. Furthermore, We have successfully found out a novel gene regulating aureobasidin sensitivity from another fungus being

sensitive to aureobasidin by using a DNA fragment of the above-mentioned gene as a probe. In addition, We have clarified that the gene regulating aureobasidin sensitivity is essentially required for the growth of the cells and found out that the detection of this gene or a protein which is a gene product thereof with an antibody enables the diagnosis of diseases caused by these cells, for example, mycoses induced by fungi, and that an antisense DNA or an antisense RNA, which inhibits the expression of the gene regulating aureobasidin sensitivity being characteristic to the cells, is usable as a remedy for diseases caused by these cells, for example, mycoses induced by fungi, thus completing the present invention.

That is to say, pathogenic fungi listed in Tables 1 and 2 and fungi and mammalian cells listed in Table 3, each having a sensitivity to aureobasidin, each carries a protein regulating aureobasidin sensitivity and a gene coding for this protein. The term "a protein regulating aureobasidin sensitivity" as used herein means a protein which is contained in an organism having a sensitivity to aureobasidin. This protein is required for the expression of the sensitivity or resistance to aureobasidin. As a matter of course, a protein having 35% or more homology with the above-mentioned protein and having a similar function is also a member of the protein regulating aureobasidin sensitivity according to the present invention. Furthermore, proteins obtained by

modifying these proteins by the genetic engineering procedure are members of the protein regulating aureobasidin sensitivity according to the present invention. A gene regulating aureobasidin sensitivity means a gene which codes for such a protein regulating aureobasidin sensitivity as those described above and involves both of sensitive genes and resistant genes.

The first invention of the present invention relates to a gene regulating aureobasidin sensitivity. This gene can be isolated in the following manner. First, aureobasidin sensitive cells (a wild-type strain) is mutagenized to thereby induce a resistant strain. From chromosome DNA or cDNA of this resistant strain, a DNA library is prepared and a gene capable of confering a resistance (a resistant gene) is cloned from this library. Then a DNA library of a wild strain is prepared and a DNA molecule being hybridizable with the resistant gene is isolated from this library and cloned. Thus a sensitive gene can be isolated.

The mutagenesis is performed by, for example, treating with a chemical such as ethylmethane sulfonate (EMS) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or by ultraviolet or other radiation. The cell that has acquired the resistance can be screened by culturing the mutagenized cells in a nutritional medium containing aureobasidin at an appropriate concentration under appropriate conditions. The resistant strain thus

obtained may vary depending on the method and conditions selected for the mutagenesis. Also, strains differing in the extent of resistance from each other can be separated by changing the aureobasidin concentration or a temperature-sensitive resistant strain can be isolated by changing the temperature in the step of screening. There are a number of mechanisms of resistance to aureobasidin. Accordingly, a number of resistant genes can be isolated by genetically classifying these various resistant strains. In the case of a yeast, the classification may be performed by the complementation test. Namely, resistant strains are prepared from haploid cells. Next, diploid cells can be obtained by crossing resistant strains differing in mating type from each other. Then spores formed from these diploids are examined by the tetrad analysis.

As typical examples of the genes regulating aureobasidin sensitivity(named aur) according to the present invention, aur1 and aur2 genes may be cited. Typical examples of the aur1 gene include spaur1 gene isolated from Schizo. pombe and scaur1 gene isolated from S. cerevisiae, while typical examples of the aur2 gene include scaur2 gene isolated from S. cerevisiae. Now, resistant genes (spaur1^R, scaur1^R and scaur2^R) isolated from resistant mutants by the present inventors and sensitive genes (spaur1^S, scaur1^S and scaur2^S) isolated from sensitive wild-type strains will be described.

Fig. 1 shows a restriction enzyme map of the genes spaur1^R and spaur1^s regulating aureobasidin sensitivity, Fig. 2 shows a restriction enzyme map of scaur1^R and scaur1^s and Fig. 3 shows a restriction enzyme map of scaur2^R and scaur2^s.

Schizo. pombe, which is sensitive to aureobasidin, is mutagenized with EMS and a genomic library of the resistant stain thus obtained is prepared. From this library, a DNA fragment containing a resistant gene (spaur1^R) and having the restriction enzyme map of Fig. 1 is isolated. This gene has a nucleotide sequence represented by SEQ ID No. 1 in Sequence Listing. The amino acid sequence of a protein encoded by this gene, which is estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 2 in Sequence Listing. By the hybridization with the use of this resistant gene as a probe, a DNA fragment containing a sensitive gene (spaur1^s) and having the restriction enzyme map of Fig. 1 is isolated from a sensitive strain. This gene has a nucleotide sequence represented by SEQ ID No. 3 in Sequence Listing. The amino acid sequence of a protein encoded by this gene, which is estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 4 in Sequence Listing. A comparison between the sequences of SEQ ID No. 3 and SEQ ID No. 1 reveals that a mutation from G to T occurs at the base at the position 1053, while a comparison between the sequences of SEQ ID No. 4 and SEQ ID No. 2 reveals

that glycine at the residue 240 is converted into cysteine at the amino acid level, thus giving rise to the resistance.

Also, S. cerevisiae, which is sensitive to aureobasidin, is mutagenized with EMS and genomic libraries of two resistant strains thus obtained are prepared. From one of these libraries, a DNA fragment containing a resistant gene ($scaur1^R$) as a dominant mutant and having the restriction enzyme map of Fig. 2 is isolated, while a DNA fragment containing a resistant gene ($scaur2^R$) and having the restriction enzyme map of Fig. 3 is isolated from another library.

The nucleotide sequence of the coding region for the protein of the $scaur1^R$ gene is the one represented by SEQ ID No. 5 in Sequence Listing. The amino acid sequence of the protein encoded by this gene, which is estimated on the basis of the above nucleotide sequence, is the one represented by SEQ ID No. 6 in Sequence Listing. By the hybridization with the use of this resistant gene $scaur1^R$ as a probe, a DNA fragment containing a sensitive gene ($scaur1^S$) and having the restriction enzyme map of Fig. 2 is isolated from a sensitive strain. This gene has a nucleotide sequence represented by SEQ ID No. 7 in Sequence Listing. The amino acid sequence of a protein encoded by this gene, which is estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 8 in Sequence Listing. A comparison between the sequences of SEQ ID No. 7 and SEQ ID No. 5 reveals that a

mutation from T to A occurs at the base at the position 852, while a comparison between the sequences of SEQ ID No. 8 and SEQ ID No. 6 reveals that phenylalanine at the residue 158 is converted into tyrosine at the amino acid level, thus giving rise to the resistance. The spaur1 gene has a 58% homology with the scaur1 gene at the amino acid level. Thus it is obvious that they are genes coding for proteins having similar functions to each other. When genes and proteins being homologous in sequence with the spaur1 and scaur1 genes and with the proteins encoded thereby are searched from a data base, none having a homology of 35% or above is detected. Accordingly, it is clear that these genes and the proteins encoded thereby are novel molecules which have never been known hitherto.

By the hybridization with the use of the DNA fragment of the resistant gene scaur2^R as a probe, a DNA fragment containing a sensitive gene (scaur2^S) and having the restriction enzyme map of Fig. 3 is isolated from a sensitive strain.

The nucleotide sequence of this sensitive gene is the one represented by SEQ ID No. 9 in Sequence Listing and the amino acid sequence of the protein encoded by this gene, which is estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 10 in Sequence Listing. As the result of the homology search with the scaur2^s gene and the protein encoded thereby, it has been found out that cystic fibrosis transmembrane conductance regulator

(CFTR) of mammals alone has a homology as low as 31%. Compared with this CFTR, however, the part having a high homology is limited to the region around the domain of the nucleotide binding. It is therefore obvious that the protein encoded by the scaur2^s gene is a protein which is completely different from CFTR in function and has never been known hitherto.

In order to prove the importance of the aur1 gene in the growth of cells, genes for disrupting the aur1 as shown in Fig. 4 and Fig. 5, in which genes coding for orotidine-5'-phosphate decarboxylase (ura4$^+$ in the case of Schizo. pombe, while URA3 in the case of S. cerevisiae) have been introduced midway in the aur1 gene, are prepared. When these aur1 disrupted genes are introduced into Schizo. pombe and S. cerevisiae respectively, the cells having the aur1 disrupted genes cannot grow at all. Thus it has been revealed that these genes and the proteins encoded thereby are essentially required for the growth of the yeast cells.

As the above examples clearly show, a gene regulating aureobasidin sensitivity can be isolated by using a organism having sensitivity to aureobasidin as a starting material and by carrying out the cloning with the use of various mutagenesis methods and/or screening methods depending on the organisms or the methods. Also, genes being hybridizable with the above-mentioned genes are involved in the scope of the first invention of the present invention. A gene

regulating aureobasidin sensitivity can be isolated by the following method. The genomic DNA library of an organism having sensitivity to aureobasidin is integrated into, for example, a high-expression vector of a yeast and transformed into the yeast. Then a clone having aureobasidin resistance is selected from the transformants and DNA is recovered from this clone. Thus the resistant gene can be obtained. As a matter of course, genes obtained by modifying some part of the gene regulating aureobasidin sensitivity thus obtained by some chemical or physical methods are involved in the scope of the first invention of the present invention.

The second invention of the present invention relates to a process for cloning a gene regulating aureobasidin sensitivity which is characterized by using the gene regulating aureobasidin sensitivity of the first invention of the present invention or a part thereof as a probe. Namely, by screening by the hybridization method or the polymerase chain reaction (PCR) method with the use of a part (consisting of at least 15 oligonucleotides) or the whole of the gene as obtained above, a gene coding for a protein having a similar function can be isolated.

For example, a pair of primers of SEQ ID No. 11 and SEQ ID No. 12 in Sequence Listing are synthesized on the basis of the DNA nucleotide sequence of the spaur1^{R} gene represented by SEQ ID No. 1. Then PCR is performed by using cDNA of C. albicans, which is a

pathogenic fungus, as a template with the use of the above-mentioned primers. The PCR is carried out and the PCR products are electrophoresed on an agarose gel and stained with ethidium bromide. In Fig. 6, the lanes 1, 2 and 3 show the results obtained by using cDNA of C. albicans, cDNA of S. cerevisiae and cDNA of Schizo. pombe as a template, respectively. As shown in Fig.6, a certain DNA fragment is specifically amplified.

By screening the genomic DNA library of C. albicans with the use of this DNA fragment as a probe, a DNA molecule having a gene (caaur1), which has the same function as that of the spaur1 and scaur1 genes and having the restriction enzyme map of Fig. 7 is obtained. The nucleotide sequence of this caaur1 gene is the one represented by SEQ ID No. 13 in Sequence Listing and the amino acid sequence of the protein encoded by this gene, which has been estimated on the basis of the above nucleotide sequence, is the one represented by SEQ ID No. 14 in Sequence Listing. It has a high homology with the proteins encoded by the spaur1 and scaur1 genes.

By screening the genomic DNA library of C. albicans with the use of a DNA fragment comprising the whole length or a part of the scaur2^{s} gene represented by SEQ ID No. 9 in Sequence Listing as a probe, a DNA fragment containing gene (caaur2), which has the same function as that of the scaur2 gene, and having the restriction enzyme map of Fig. 8 is obtained. The

nucleotide sequence of a part of this caaur2 gene is the one represented by SEQ ID No. 15 in Sequence Listing and the amino acid sequence of the region encoded by this gene, which has been estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 16 in Sequence Listing. It has a high homology with the corresponding region of the protein encoded by the scaur2 gene.

The third invention of the present invention relates to an oligonucleotide comprising 15 or more bases which serves as the above-mentioned nucleic acid probe and is hybridizable with the gene regulating aureobasidin sensitivity, for example, the DNA fragment having the restriction enzyme map as shown in Fig. 1, Fig. 2 or Fig. 3. This nucleic acid probe is usable in, for example, the hybridization in situ, the identification of a tissue wherein the above-mentioned gene can be expressed, and the confirmation of the presence of a gene or mRNA in various vital tissues. This nucleic acid probe can be prepared by ligating the above-mentioned gene or a gene fragment to an appropriate vector, introducing it into a bacterium, allowing it to replicate in the bacterium, extracting from a disrupted cell suspension, cleaving with a restriction enzyme capable of recognizing the vector-ligating site, electrophoresing and then excising from the gel. Alternatively, this nucleic acid probe can be constructed by the chemical synthesis with the use of a DNA synthesizer or gene amplification techniques by

PCR on the basis of the nucleotide sequence of SEQ ID. Nos. 1, 3, 5, 7, 9, 13, 15 or 21 in Sequence Listing. This nucleic acid probe can be labeled with a radioisotope or a fluorescent substance to thereby elevate the detection sensitivity at the use.

The fourth invention of the present invention relates to an antisense DNA of the above-mentioned gene regulating aureobasidin sensitivity, while the fifth invention of the present invention relates to an antisense RNA thereof. The introduction of the antisense DNA or antisense RNA into cells makes it possible to control the expression of the gene regulating aureobasidin sensitivity.

As examples of the antisense DNA to be introduced, antisense DNAs corresponding to the genes regulating aureobasidin sensitivity of SEQ ID Nos. 1, 3, 5, 7, 9, 13, 15 or 21 in Sequence Listing and some parts thereof may be cited. SEQ ID No. 17 in Sequence Listing shows an example of this antisense DNA. It represents the sequence of an antisense DNA of the gene regulating aureobasidin sensitivity of SEQ ID No. 1 in Sequence Listing. A fragment obtained by appropriately cleaving some part of such an antisense DNA, and a DNA synthesized depending on such an antisense DNA sequence may be used as the antisense DNA.

As examples of the antisense RNA to be introduced, antisense RNAs corresponding to the genes regulating aureobasidin sensitivity of SEQ ID Nos. 1, 3, 5, 7, 9,

13, 15 or 21 in Sequence Listing and some parts thereof may be cited. SEQ ID No. 18 in Sequence Listing shows an example of this antisense RNA. It represents the sequence of an antisense RNA of the gene regulating aureobasidin sensitivity of SEQ ID No. 1 in Sequence Listing. A fragment obtained by appropriately cleaving some part of such an antisense RNA, an RNA synthesized depending on such an antisense RNA sequence, and an RNA prepared with RNA polymerase in an *in vitro* transcription system by using the DNA corresponding to the gene regulating aureobasidin sensitivity of SEQ ID No. 1 or SEQ ID No. 3 in Sequence Listing or a part thereof may be used as the antisense RNA.

These antisense DNA and antisense RNA may be chemically modified so as to prevent degradation *in vivo* or to facilitate passage through a cell membrane. A substance capable of inactivating mRNA, for example, ribozyme may be linked thereto. The antisense DNA and antisense RNA thus prepared are usable in the treatment of various diseases such as mycoses accompanied by an increase in the amount of mRNA coding for a protein regulating aureobasidin sensitivity.

The sixth invention of the present invention relates to a recombinant plasmid having a gene coding for a protein regulating aureobasidin sensitivity being integrated into an appropriate vector. For example, a plasmid, in which a gene regulating

aureobasidin sensitivity gene has been integrated into an appropriate yeast vector, is highly useful as a selection marker gene, since a transformant can be easily selected thereby with the guidance of the chemical resistance by using aureobasidin.

Also, the recombinant plasmid can be stably carried by, for example, Escherichia coli. Examples of vectors which are usable in this case include pUC118, pWH5, pAU-PS, Traplex119 and pTV118. pAU-PS having the spaur1^s gene integrated therein is named pSPAR1. pWH5 having the spaur1^s gene integrated therein is named pSCAR1. pWH5 having the scaur2^R gene integrated therein is named pSCAR1. Traplex119 vector having the caaur1 gene integrated therein is named pCAAR1. pTV118 vector having a part of the caaur2 gene integrated therein is named pCAAR2N. Each of these recombinant plasmids is transformed into E. coli. It is also possible to express these plasmids in an appropriate host. Such a gene is reduced exclusively into the open reading frame (ORF) to be translated into a protein by cleaving with an appropriate restriction enzyme, if necessary, and then bound to an appropriate vector. Thus an expression recombinant plasmid can be obtained. When E. coli is used as the host, plasmids such as pTV118 may be used as a vector for the expression plasmid. When a yeast is used as the host, plasmids such as pYES2 may be used as the vector. When mammalian cells are used as the host, plasmids such as pMAMneo may be used as the vector.

The seventh invention of the present invention relates to a transformant having the above-mentioned recombinant plasmid which has been introduced into an appropriate host. As the host, E. coli, yeasts and mammalian cells are usable. E. coli JM109 transformed by pSPAR1 having the spaur1^{s} gene integrated therein has been named and designated as Escherichia coli JM109/pSPAR1 and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken 305, JAPAN) on 13 April 1993, in accordance with the Budapest Treaty under the accession number FERM BP-4485. E. coli HB101 transformed by pSCAR1 having the scaur1^{s} gene integrated therein has been named and designated as Escherichia coli HB101/pSCAR1 and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on 13 April 1993 in accordance with the Budapest Treaty under the accession number FERM BP-4483. E. coli HB101 transformed by pSCAR2 having the scaur2^{R} gene integrated therein has been named and designated as Escherichia coli HB101/pSCAR2 and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on 13 April 1993 in accordance with the Budapest Treaty under the accession number FERM BP-4484. E. coli HB101 transformed by pCCAR1 having the caaur1^{s} gene integrated therein has been named and designated as Escherichia coli HB101/pCAAR1 and deposited at

National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on 1 December 1993 in accordance with the Budapest Treaty under the accession number FERM BP-4482. E. coli HB101 transformed by pCAAR2N having a part of the caaur2 gene integrated therein has been named and designated as Escherichia coli HB101/pCAAR2N and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on 1 December 1993 in accordance with the Budapest Treaty under the accession number FERM BP-4481.

A transformant capable of expressing a protein regulating aureobasidin sensitivity can be obtained by transforming a expression recombinant plasmid into an appropriate host, as described above. For example, a yeast having a recombinant plasmid as shown in Fig. 9 introduced thereinto is usable for this purpose.

The eighth invention of the present invention relates to a process for producing a protein regulating aureobasidin sensitivity which comprises incubating a transformant according to the sixth invention of the present invention, which contains a gene coding for this protein, in an appropriate nutritional medium, allowing the expression of the protein, then recovering the protein from the cells or the medium and purifying the same. For the expression of the gene coding for this protein, E. coli, a yeast or mammalian cells are employed as a host. When the yeast having the recombinant plasmid of Fig. 9 is

incubated in a medium containing galactose, for example, the protein regulating aureobasidin sensitivity which is encoded by the $scaur1^s$ gene can be expressed.

The ninth invention of the present invention relates to an isolated protein regulating aureobasidin sensitivity. As examples of such a protein, those encoded by the above-mentioned spaur1, scaur1, scaur2, caaur1 and caaur2 genes can be cited.

The $spaur1^s$ gene codes for a protein having an amino acid sequence represented by SEQ ID No. 4 in Sequence Listing, while the $scaur1^s$ gene codes for a protein having an amino acid sequence represented by SEQ ID No. 8 in Sequence Listing. By the northern hybridization with the use of a DNA fragment of the spaur1 gene as a probe, mRNAs are detected from a sensitive strain (Fig. 10). Thus the expression of the spaur1 gene is confirmed.

Fig. 10 is an autoradiogram showing the results of the northern hybridization wherein mRNAs obtained from cells of a sensitive strain of Schizo. pombe in the logarithmic growth phase (lane 1), cells of a resistant strain in the logarithmic growth phase (lane 2), cells of the sensitive strain in the stationary phase (lane 3) and cells of the resistant strain in the stationary phase (lane 4) are electrophoresed on a 1.2% agarose gel containing formaldehyde.

The tenth invention of the present invention relates to an antibody against the above-mentioned

protein regulating aureobasidin sensitivity. For example proteins having amino acid sequences of SEQ ID Nos. 2, 4, 6, 8, 10, 14, 16 or 22 in Sequence Listing and peptides comprising some parts of these amino acid sequences may be used as an antigen. The former antigens can be prepared through the expression in a transformant followed by purification, while the latter antigens can be synthesized on, for example, a marketed synthesizer. The antibody is produced by the conventional method. For example, an animal such as a rabbit is immunized with the above-mentioned protein or a peptide fragment together with an adjuvant to thereby give a polyclonal antibody. A monoclonal antibody can be produced by fusing antibody-producing B cells, which have been obtained by immunizing with an antigen, with myeloma cells, screening hybridomas producing the target antibody, and incubating these cells. As will be described hereinafter, these antibodies are usable in the treatment and diagnosis for animal and human diseases in which the above-mentioned proteins participate, such as mycoses.

For example, a peptide corresponding to the part of the 103- to 113-positions in the amino acid sequence of SEQ ID No. 8 is synthesized on a synthesizer and then bound to a carrier protein. Then a rabbit is immunized therewith and thus a polyclonal antibody is obtained. In the present invention, keyhole limpet hemocyanin (KLH) is used as the carrier protein. Alternatively, bovine serum albumin and

ovalbumin are usable therefor.

The eleventh invention of the present invention relates to a process for detecting a protein regulating aureobasidin sensitivity by using the above-mentioned antibody. The detection can be carried out by detecting the binding of the antibody to the protein or measuring the amount of binding. For example, the protein or the cells producing the same can be detected by treating with a fluorescence-labeled antibody and then observing under a fluorescence microscope. The amount of the antibody bound to the protein can be measured by various known methods. For example, S. cerevisiae cells are stained by the immunofluorescent antibody technique by using the above-mentioned antibody and a secondary antibody such as FITC-labeled antirabbit antibody. Thus it is clarified that the protein encoded by the scaur1 gene is distributed all over the cells. Further, a yeast having the recombinant plasmid of Fig. 9 introduced thereinto is incubated in a medium containing glucose or galactose. The cells thus obtained are disrupted with glass beads and proteins are solubilized. Then these proteins are separated by SDS-polyacrylamide gel electrophoresis(SDS-PAGE) and the western blotting is carried out in the conventional manner by using the above-mentioned polyclonal antibody and peroxidase-labeled anti-rabbit antibody. Consequently, the protein encoded by the scaur1 gene can be detected, as Fig. 11 shows.

Fig. 11 shows the results of the western blotting wherein the proteins prepared from the cells obtained by the incubation in the presence of glucose (lane 1) or galactose (lane 2) are subjected to SDS-PAGE. A main band binding to the polyclonal antibody of the present invention is detected at around 38 kDa.

The twelfth invention of the present invention relates to a process for detecting a gene regulating aureobasidin sensitivity, for example, mRNA at the expression of a protein, by using the above-mentioned oligonucleotide as a nucleic acid probe. This process is applicable to the diagnosis for various diseases, including mycoses, associated with an abnormal amount of mRNA coding for the protein. For example, nucleic acids are precipitated from disrupted cells and mRNA is hybridized with a radioisotope-labeled nucleic acid probe on a nitrocellulose membrane. The amount of binding can be measured by autoradiography (Fig. 10) or with a scintillation counter.

The thirteenth invention of the present invention relates to a process for efficient screening of a novel antimycotic by using the transformant of the seventh invention of the present invention or the protein regulating aureobasidin sensitivity of the ninth invention of the present invention. For example, a drug exerting its effect on the protein or the gene of the present invention can be efficiently found out through a comparison of the activity on a transformant

containing a sensitive gene with the activity on a transformant containing a resistant gene or a comparison between the activities on transformants differing in expression level from each other. Also, the screening can be efficiently carried out by measuring the affinity for the protein of the present invention, for example, the activity of inhibiting the binding of radiolabeled-aureobasidin to the protein.

[Brief Description of the Drawings]

[Fig. 1]

Restriction enzyme map of the genes $spaur1^R$ and $spaur1^S$ regulating aureobasidin sensitivity.

[Fig. 2]

Restriction enzyme map of $scaur1^R$ and $scaur1^S$.

[Fig. 3]

Restriction enzyme map of $scaur2^R$ and $scaur2^S$.

[Fig. 4]

Structure of a DNA for disrupting the _Schizo. pombe_ $spaur1^S$ gene.

[Fig. 5]

Structure of a DNA for disrupting the _S. cerevisiae_ $scaur1^S$ gene.

[Fig. 6]

Results of the detection of the aur1 gene caaur1 carried by _C. albicans_ by the PCR method.

[Fig. 7]

Restriction enzyme map of the caaur1 gene carried by _C. albicans_.

[Fig. 8]

Restriction enzyme map of the caaur2 gene.

[Fig. 9]

Structure of a plasmid YEpSCARW3 for expressing the scaur1 gene.

[Fig. 10]

Results of the northern hybridization of the spaur1 gene of Schizo. pombe.

[Fig. 11]

Results of the detection of the scaur1 protein by using an antibody.

[Fig. 12]

Restriction enzyme map of pAR25.

[Examples]

To further illustrate the present invention in greater detail, the following Examples will be given. However it is to be understood that the present invention is not restricted thereto.

Example 1: Cloning of a gene regulating aureobasidin sensitivity originating in fission yeast Schizo. pombe

1-a) Separation of aureobasidin-resistant mutant of Schizo. pombe

About 1 x 10^8 cells of a Schizo. pombe haploid cell strain JY745 (mating type h^-, genotype ade6-M210, leu1, ura4-D18) exhibiting a sensitivity to aureobasidin at a concentration of 0.08 μg/ml were suspended in 1 ml of a phosphate buffer containing

0.9% NaCl. Then the cells were mutagenized with EMS at a final concentration of 3% at 30°C for 90 minutes. After neutralizing by adding 8 ml of 5% sodium thiosulfate, the cells thus treated were harvested by centrifugation (2500 r.p.m., 5 minutes), washed twice with 6 ml of physiological saline and then suspended in 2 ml of a YEL medium (3% of glucose, 0.5% of yeast extract). The suspension was incubated at 30°C for 5 hours under stirring and then spreaded on a YEA plate (the YEL medium containing 1.5% of agar) containing 5 μg/ml of aureobasidin A. After incubating at 30°C for 3 to 4 days, two or three aureobasidin-resistant colonies were formed per 1×10^8 cells. After carrying out the mutagenesis several times, five clone mutants, i.e., THR01, THR04, THR05, THR06 and THR07 were obtained. These mutants were resistant to more than 25 μg/ml of aureobasidin A but the same as the parent strain in the sensitivity to cycloheximide and amphotericin B. Therefore it is estimated that they are not mutants having a multiple drug resistance but ones having a resistance specific to aureobasidin.

1-b) Genetic analysis

Each of the above-mentioned resistant strains THR01, THR04, THR05, THR06 and THR07 was crossed with normal cells of Schizo. pombe LH121 strain (mating type h^+, genotype ade6-M216, ura4-D18) differing in mating type. Diploid cells obtained were examined about the resistance to aureobasidin. Similar to the

resistant strains, the five diploids formed by crossing the resistant strains with the normal one were resistant to 25 μg/ml of aureobasidin A, thus proving that these resistant mutations were dominant. To perform the tetrad analysis, the above-mentioned diploids were subsequently inoculated on an MEA medium (3% of malt extract, 2.5% of agar) for sporulation and incubated at 25°C for 2 days. Prior to the meiosis, the diploid cells replicated DNA on the MEA medium and then underwent the meiosis to form asci each containing four ascospores of the haploid. These spores were separated with a micromanipulator and allowed to germinate on the YEA plate, followed by the formation of colonies. Then the resistance to aureobasidin of these colonies was examined. Among four spores contained in an ascus, the separation of the sensitivity versus the resistance showed 2 : 2. This result indicates that the aureobasidin resistant mutation was induced by a mutation in single gene. Further, the complementation test was performed in order to confirm whether the resistant genes of the above-mentioned five mutants were identical with each other or not. For example, a mutant of the mating type h^+, which had been obtained by crossing the mutant THR01 with the LH121 strain in the above tetrad analysis, was crossed with another variant THR04 (mating type h^-) on the MEA plate as described above and, after sporulation, the tetrad analysis was carried out. As a result, all of the colonies formed

from four ascospores showed resistance to aureobasidin, which indicates that the mutational genes of THR01 and THR04 are the same with each other. Similarly, the five mutants were examined and it was thus found out that all mutations occurred on the same gene. This gene regulating aureobasidin sensitivity is named spaur1, the normal gene (sensitive gene) is named $spaur1^{S}$ and the mutational gene (resistant gene) is named $spaur1^{R}$.

1-c) Preparation of genomic library of aureobasidin resistant strain

Genomic DNA was extracted and purified from the aureobasidin resistant strain THR01 by the method of P. Philippsen et al. [Methods in Enzymology, 194, 169 - 175 (1991)]. The purified genomic DNA (8 μg) was partially digested by treating with 5 U of a restriction enzyme *Hin*dIII at 37°C for 10 minutes, deproteinized with phenol/chloroform and precipitated with ethanol. The partially digested DNA was electrophoresed on a 0.8% agarose gel and DNA in the region of 3 to 15 kb was extracted. The DNA thus obtained was ligated with a yeast-*E. coli* shuttle vector pAU-PS (2 μg) which had been completely digested with *Hin*dIII by using a DNA ligation kit (manufactured by Takara Shuzo Co., Ltd.) and then transformed into *E. coli* HB101. Thus a genomic library of the aureobasidin resistant strain was formed. *E. coli* containing this genomic library was incubated in 50 ml of an LB medium (1% of bacto trypton, 0.5% of

bacto yeast extract, 0.5% of sodium chloride) containing 100 μg/ml of ampicillin and 25 μg/ml of tetracycline at 37°C overnight. Then a plasmid was recovered and purified from the E. coli cells.

1-d) Expression and cloning of aureobasidin resistant gene spaur1^R

The plasmid originating in the genomic library of the aureobasidin resistant strain as prepared above was transformed into a strain Schizo. pombe JY745 by the method of Okazaki et al. [Nucleic Acid Research, 18, 6485 - 6489 (1990)]. The transformed cells were spreaded on a minimum medium SD plate [0.67% of yeast nitrogen base without amino acids (manufactured by Difco), 2% of glucose, 2% of agar] containing 75 μg/ml of adenine sulfate and 50 μg/ml of leucine. After incubating at 30°C for 3 to 4 days, the colonies thus formed were replicated onto an SD plate containing 5 μg/ml of aureobasidin A, 75 μg/ml of adenine sulfate and 50 μg/ml of leucine. It is conceivably that a colony propagated on this plate may have the plasmid containing the aureobasidin resistant gene. This colony was inoculated into 5 ml of a liquid SD medium containing 75 μg/ml of adenine sulfate and 50 μg/ml of leucine. After incubating at 30°C for 2 days, the plasmid was recovered from the propagated cells by the method of I. Hagan et al. [J. Cell Sci., 91, 587 - 595 (1988)]. Namely, the cells were harvested from the culture (5 ml) by centrifugation and then suspended in 1.5 ml of 50 mM citrate/phosphate buffer containing

1.2 M of sorbitol and 2 mg/ml of Zymolyase. Then the suspension was maintained at 37 °C for 60 minutes. The cells were collected by centrifuging at 3,000 r.p.m. for 30 seconds and suspended in 300 μl of a TE [10 mM of Tris-HCl, pH 8, 1 mM of EDTA] solution. After adding 35 μl of 10% SDS, the mixture was maintained at 65°C for 5 minutes. After adding 100 μl of 5 M potassium acetate, the mixture was allowed to stand in ice for 30 minutes. Then it was centrifuged at 10,000 r.p.m. at 4°C for 10 minutes and a plasmid DNA was purified from the supernatant by using EASYTRAP™ (manufactured by Takara Shuzo Co., Ltd.).

This plasmid was transformed into E. coli HB101 and a plasmid DNA was prepared from E. coli colonies formed on an LB medium containing ampicillin and tetracycline. This plasmid, which contained a DNA of 4.5 kb, was named pAR25. Fig. 12 shows the restriction enzyme map of the DNA of 4.5 kb in pAR25. To specify the gene region, HindIII fragments or SacI fragments of various sizes were subcloned into the pAU-PS vector. These DNAs were transformed into normal JY745 cells by the above-mentioned method of Okazaki et al. and the acquisition of aureobasidin resistance was examined. As a result, it is revealed that a HindIII-SacI 2.4 kb DNA fragment contains the $spaur1^R$ gene. The restriction enzyme map of this DNA segment containing the aureobasidin resistant gene $spaur1^R$ is shown in Fig. 1. This fragment was cloned into a pUC118 vector (named pUARS2R) and then the DNA

nucleotide sequence was identified (SEQ ID No. 1 in Sequence Listing). From this nucleotide sequence, it is revealed that the spaurl^{R} gene code for a protein having an amino acid sequence represented by SEQ ID No. 2 in Sequence Listing.

1-e) Cloning of aureobasidin sensitive gene spaurl^{S}

By the same method as the one employed in the above c), genomic DNA was extracted and purified from normal cells. After partially digesting with HindIII, a genomic library of the normal cells was constructed. An E. coli stock containing this library DNA was spreaded on an LB-agar medium containing ampicillin and tetracycline and incubated overnight at 37°C. The colonies thus formed were transferred onto a nylon membrane (Hybond™-N, manufactured by Amersham) and the colony hybridization was performed.

As a probe, the above-mentioned DNA fragment (2.4 kb) obtained by cleaving the spaurl^{R} gene with HindIII-SacI and labeled with [α-^{32}p] dCTP by using a random primer DNA labeling kit (manufactured by Takara Shuzo Co., Ltd.) was used. As the results of screening of 5 x 10^{4} colonies, five clones being hybridizable with the probe were obtained. Plasmids were purified from E. coli cells of these five clones. As the result of the cleavage with restriction enzymes, it was found out that all of these clones contained the same DNA fragment of 4.5 kb (named pARN1). The restriction enzyme map of the DNA of 4.5 kb in pARN1 was identical with that of pAR25 shown in

Fig. 10. Therefore, a HindIII-SacI 2.4 kb DNA fragment which was a region containing the spaur1^{s} gene was prepared from pARN1. Then it was cloned into the pAU-PS vector and this plasmid was named pSPAR1.

By using this plasmid pSPAR1, a strain E. coli JM109 was transformed and the transformant thus obtained was named and designated as Escherichia coli JM109/pSPAR1. It has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4485. This DNA fragment containing the aureobasidin sensitive gene spaur1^{s} had the restriction enzyme map shown in Fig. 1 and the DNA nucleotide sequence thereof was the one represented by SEQ ID No. 3 in Sequence Listing. Based on this nucleotide sequence, it has been revealed that the spaur1^{s} gene codes for a protein having the amino acid sequence represented by SEQ ID No. 4 in Sequence Listing and, when compared with the resistant gene spaur1^{R}, the amino acid at the residue 240 has been changed from glycine into cysteine.

Example 2: Cloning of aureobasidin sensitive genes scaur1 and scaur2 originating in budding yeast S. cerevisiae

2-a) Separation of aureobasidin resistant mutant of S. cerevisiae

A strain S. cerevisiae DKD5D (mating type a,

genotype leu2-3 112, trp1, his3) having a sensitivity to aureobasidin at a concentration of 0.31 μg/ml was mutagenized with EMS in the same manner as the one employed in the case of Schizo. pombe. Then resistant mutants were separated on an agar plate of a complete nutritional medium YPD (1% of yeast extract, 2% of polypeptone, 2% of glucose) containing 5 μg/ml or 1.5 μg/ml of aureobasidin A. After repeating the mutagenesis several times, 34 mutant clones were obtained. These mutants were resistant to more than 25 μg/ml of aureobasidin A and estimated as having not a multiple drug resistance mutation but a aureobasidin-specific resistance mutation.

2-b) Genetic analysis

Similar to the above-mentioned case of Schizo. pombe, the genetic analysis using the tetrad analysis and the complementation test was performed. As a result, the genes could be classified into two types. These genes regulating aureobasidin sensitivity were named scaur1 and scaur2, the resistant genes isolated from the resistant mutant were named $scaur1^R$ and $scaur2^R$, and the sensitive genes isolated from the sensitive wild-type strain were named $scaur1^s$ and $scaur2^s$, respectively.

The R94A strain had a gene with dominant mutation ($scaur1^R$). It has been further clarified that the scaur1 gene is located in the neighborhood of the met14 gene of the eleventh chromosome.

2-c) Preparation of genomic library of aureobasidin resistant strain having aureobasidin resistant gene scaur1^R

Genomic DNA was extracted and purified from the aureobasidin resistant strain R94A by the above-mentioned method of P. Philippsen et al. The purified genomic DNA (8 μg) was partially digested by treating with 5 U of a restriction enzyme HindIII at 37°C for 10 minutes, deproteinized with phenol/chloroform and precipitated with ethanol. The partially digested DNA thus obtained was electrophoresed on a 0.8% agarose gel and DNA in the region of 3 to 15 kb was extracted. The DNA thus obtained was ligated with a yeast-E. coli shuttle vector pWH5 (2 μg) which had been completely digested with HindIII by using a DNA ligation kit and then transformed into E. coli HB101. Thus a genomic library was formed. E. coli containing this genomic library was cultured in 50 ml of an LB medium containing ampicillin and tetracycline at 37°C overnight. Then a plasmid was recovered and purified from the E. coli cells.

2-d) Expression and cloning of aureobasidin resistant gene scaur1^R

The above-mentioned genomic library of the R94A strain was transformed into S. cerevisiae SH3328 (mating type α, genotype ura3-52, his4, thr4, leu2-3 • 112) in accordance with the method of R.H. Schiestl et al. [Current Genetics, 16, 339 - 346 (1989)]. The transformed cells were spread on a

minimum medium SD plate [0.67% of yeast nitrogen base without amino acids, 2% of glucose, 2% of agar] containing 25 μg/ml of uracil, 35 μg/ml of histidine and 500 μg/ml of threonine. After incubating at 30°C for 3 to 4 days, the colonies thus formed were replicated onto a YPD agar plate containing 1.5 μg/ml of aureobasidin A. A colony thus formed was inoculated into 5 ml of a liquid YPD medium. After incubating at 30°C for 2 days, a plasmid DNA was recovered from the propagated cells by the above-mentioned method of I. Hagan et al. This plasmid was transformed into a yeast again and it was confirmed that the obtained transformant had acquired aureobasidin resistance. This plasmid, which contained a DNA of 3.5 kb, was named pWTCR3. Neither the DNA fragment of 2.0 kb nor the DNA fragment of 1.5 kb obtained by cleaving with HindIII exhibited any aureobasidin resistant activity alone. Thus it is confirmed that the gene is contained in the DNA fragment of 3.5 kb. Fig. 2 shows the restriction enzyme map of this DNA fragment of 3.5 kb containing the aureobasidin resistant gene $scaur1^R$. The HindIII fragments of 1.5 kb and 2 kb were each cloned into pUC118, followed by the determination of the DNA nucleotide sequence (SEQ ID No. 5 in Sequence Listing). From this nucleotide sequence, it has been revealed that the $scaur1^R$ gene codes for a protein having an amino acid sequence represented by SEQ ID No. 6 in Sequence Listing.

2-e) Cloning of aureobasidin sensitive gene $scaur1^S$ corresponding to aureobasidin resistant gene $scaur1^R$

By the same method as the one employed in the above Example 2-c), genomic DNA was extracted and purified from the parent strain S. cerevisiae DKD5D. After partially digesting with HindIII, the DNA was ligated with pWH5 and transformed into E. coli HB101. Thus a genomic library of the normal cells was formed. An E. coli stock containing this library DNA was spreaded on an LB agar medium containing ampicillin and tetracycline and incubated overnight at 37°C. The colonies thus formed were transferred onto a nylon membrane (Hybond™-N) and the colony hybridization was carried out. As a probe, the DNA fragment of 3.5 kb obtained in the above Example 2-d) and labeled with [α-^{32}P] dCTP by using a random primer DNA labeling kit (manufactured by Takara Shuzo Co., Ltd.) was used. As the results of screening of 2 x 10^4 colonies, seven clones being hybridizable with the probe were obtained. Plasmids were purified from E. coli cells of these clones. As the result of the cleavage with restriction enzymes, one of these clones contained a DNA fragment of 3.5 kb. This DNA fragment had the restriction enzyme map of Fig. 2 and thus judged as containing the $scaur1^S$ gene. The plasmid containing this DNA fragment was named pSCAR1, while E. coli HB101 having this plasmid introduced therein was named and designated as Escherichia coli HB101/pSCAR1. This

strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4483. The DNA fragment of 3.5 kb obtained by partially digesting pSCAR1 with *HindIII* was subcloned into pUC118 and the nucleotide sequence thereof was determined (SEQ ID No. 7 in Sequence Listing). A comparison with the resistant gene indicates that the base at the position 852 has been changed from T into A and, due to this replacement, the amino acid has been converted from phenylalanine into tyrosine (SEQ ID No. 8 in Sequence Listing).

2-f) Preparation of genomic library of aureobasidin resistant strain having aureobasidin resistant gene $scaur2^R$

A genomic library was prepared from an aureobasidin resistant strain L22-8B by the same method as the one described in Example 2-c). *E. coli* containing this genomic library was cultured in an LB medium (50 ml) containing ampicillin and tetracycline at 37°C overnight. Then plasmids were recovered and purified from the *E. coli* cells.

2-g) Expression and cloning of aureobasidin resistant gene $scaur2^R$

The above-mentioned plasmids originating in the genomic library of the L22-8B strain were transformed into *S. cerevisiae* SH3328 by the above-mentioned method of R.H. Schiestl. From the transformed

strains, an aureobasidin resistant strain was isolated. Then a plasmid DNA containing the scaur2^R gene was recovered from this transformant by the above-mentioned method of I. Hagan et al. This plasmid was transformed into a yeast again and it was confirmed that the transformant had acquired aureobasidin resistance. This plasmid, which contained a DNA of 8.5 kb, was named pSCAR2. Fig. 3 shows the restriction enzyme map of the DNA fragment of 8.5 kb containing this aureobasidin resistant gene scaur2^R. *E. coli* HB101 having this plasmid pSCAR2 introduced therein was named and designated as *Escherichia coli* HB101/pSCAR2. This strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4484. By using *Bam*HI, *Eco*RI, *Hind*III and *Pst*I, DNA fragments of various sizes were prepared and cloned into the pWH5 vector. These plasmids were transformed into *S. cerevisiae* DKD5D in accordance with the above-mentioned method of R.H. Schiestl et al. Then it was examined whether these transformants had acquired aureobasidin resistance or not. As a result, none of the transformants of the DNA fragments was a resistant one. Thus it has been clarified that the DNA fragment of the full length is necessary for the expression of the aureobasidin resistance.

2-h) Isolation of aureobasidin sensitive gene $scaur2^S$ corresponding to aureobasidin resistant gene $scaur2^R$

An E. coli stock containing the genomic library of Example 2-e) prepared from normal cells of S. cerevisiae DKD5D was spreaded on an LB agar medium containing ampicillin and tetracycline and incubated at 37°C overnight. The colonies thus formed were transferred onto a nylon membrane (Hybond™-N) and the colony hybridization was performed. As a probe the DNA fragment of 8.5 kb obtained in the above Example 2-g) and labeled with [α-^{32}P] dCTP by using a random primer DNA labeling kit was used. As the results of screening of 2 x 10^4 colonies, several clones being hybridizable with the probe were obtained. Some of these clones contained a DNA fragment of 4.6 kb while others contained a DNA fragment of 3.9 kb. From the restriction enzyme maps of these DNA fragments, it was found out that these DNA fragments were each a part of the $scaur2^S$ gene shown in Fig. 3. These DNA fragments were ligated together to thereby give a $scaur2^S$ fragments shown in Fig. 3. The DNA fragment of 8.5 kb thus obtained was subcloned into pUC118 and then the DNA nucleotide sequence was determined (SEQ ID No. 9 in Sequence Listing). Based on the nucleotide sequence of SEQ ID No. 9 in Sequence Listing, the amino acid sequence represented by SEQ ID No. 10 in Sequence Listing was estimated.

Example 3: Gene disruption test on $spaur1^s$ and $scaur1^s$ genes

3-a) Gene disruption test on $spaur1^s$ gene

In order to examine whether the aureobasidin sensitive gene $spaur1^s$ is necessary in the cell growth by the gene disruption test, the plasmid pUARS2R prepared in Example 1-d) was first cleaved with BalI and EcoT22I. After eliminating a DNA fragment of 240 bp, the residual DNA fragment was blunted by using a DNA blunting kit (manufactured by Takara Shuzo Co., Ltd.). Then this DNA was ligated with a DNA containing $ura4^+$ gene of 1.7 kb, which had been obtained by excising from a pUC8ura4 plasmid [Mol. Gen. Genet., 215, 81 - 86 (1988)] by cleaving with HindIII and blunting, to thereby give a plasmid pUARS2RBT22::ura4-1 and another plasmid pUARS2RBT22::ura4-6 in which the ura4 DNA had been inserted in the opposite direction. Both of these disrupted genes were excised from the vector pUC118 by cleaving with SacI and HindIII and ARS2RBT22::ura4-1 and ARS2RBT22::ura4-6 (Fig. 4), which were $spaur1^s$ DNA fragments containing $ura4^+$, were purified. The purified DNA fragments were transformed into diploid cells Schizo. pombe C525 (h^{90}/h^{90}, ura4-D18/ura4-D18, leu1/leu1, ade6-M210/ade6-M216) by the above-mentioned method of Okazaki et al. and then a transformant was screened on an SD agar plate containing leucine. In the transformant thus obtained, one of a pair of $spaur1^s$ genes on the chromosome had been replaced by the disrupted gene ARS2RBT22::ura4-1

or ARS2RBT22::ura4-6 introduced thereinto. These cells were allowed to undergo sporulation on a sporulation medium MEA and subjected to the tetrad analysis. As a result, it was found out that two of the four ascospores formed colonies but the residual two spores formed no colony. That is to say, the spores suffering from the replacement of the normal spaur1^s gene by the disrupted gene ARS2RBT22::ura4-1 were not propagated. It has been thus revealed that the spaur1^s gene is essentially required for the growth of the cells.

3-b) Gene disruption test on scaur1^s gene

The plasmid pSCAR1 prepared in Example 2-e) was partially digested with HindIII to thereby give a DNA fragment of 3.5 kb shown in Fig. 2. This DNA fragment was cloned into the HindIII site of pUC119 and the obtained product was named pSCAR3. The obtained pSCAR3 was cleaved with StuI and EcoT22I. After eliminating a DNA fragment of 0.3 kb, the obtained DNA was ligated with a DNA fragment (1.1 kb) of URA3 gene which had been obtained by cleaving a plasmid pYEUra3 (manufactured by Clontech Laboratories, Inc.) with HindIII and EcoRI and blunting. Thus a plasmid pUSCAR3.ST22::URA3$^+$ and another plasmid pUSCAR3.ST22::URA3A, in which the URA3 gene had been inserted in the opposite direction, were obtained. These disrupted gene were excised in the EcoRI site in the scaur1^s gene and the EcoRI site in the pUC119 vector by cleaving with EcoRI. The scaur1^s DNA

fragments containing URA3, SCAR3.ST22::URA3^{+} and SCAR3.ST22::URA3A (Fig. 5), were purified. The purified DNA fragments were transformed into diploid cells of S. cerevisiae AOD1 (mating type a/α, genotype ura3-52/ura3-52, leu2-3 112/leu2-3 112, trp1/TRP1, thr4/THR4, his4/HIS4) by the above-mentioned method of R.H. Schiestl and transformants were screened on an SD agar plate containing leucine. The transformants thus obtained were allowed to undergo sporulation on a sporulation medium SP (1% of potassium acetate, 2% of agar) and subjected to the tetrad analysis. As a result, it was found out that two of the four ascospores underwent germination and formed colonies but the residual two spores did not undergo colony formation. That is to say, the spores suffering from the replacement of the scaur1^{s} gene by the disrupted gene were not propagated. It has been thus revealed that the scaur1^{s} gene is essentially required for the growth of the cells.

Example 4: Examination on the expression of aureobasidin sensitive gene spaur1 by northern hybridization

From a normal strain or a resistant strain of Schizo. pombe, the whole RNAs were extracted and purified by the method of R. Jensen et al. [Proc. Natl. Acad. Sci. USA, 80, 3035 - 3039 (1983)]. Further, poly(A) RNA was purified by using Oligotex™-dT30 (manufactured by Takara Shuzo Co., Ltd.). The

purified poly(A)$^+$RNA (2.5 μg) was separated by the electrophoresis on a 1.2% agarose gel containing formaldehyde and transferred onto a nylon membrane (Hybond™-N). After immobilizing, the hybridization was performed with the use of a HindIII-SacI fragment (2 kb) of the spaurl^R gene labeled with [α-^{32}P]dCTP as a probe. As a result, both of the normal cells and the resistant cells showed a band of the same amount of about 2 kb. In both cases, this amount underwent no change in the logarithmic growth phase and the stationary phase (Fig. 10). Fig. 10 is an autoradiogram showing the results of the northern hybridization wherein mRNAs obtained from cells of a sensitive strain of Schizo. pombe in the logarithmic growth phase (lane 1), cells of a resistant strain in the logarithmic growth phase (lane 2), cells of the sensitive strain in the stationary phase (lane 3) and cells of the resistant strain in the stationary phase (lane 4) are electrophoresed on a 1.2% agarose gel containing formaldehyde.

Example 5: Determination of the activity of scaurl^S gene

5-a) Construction of plasmid YEpSCARW3 (Fig. 9) and YEpSCARW1

The plasmid pSCAR1 prepared in Example 2-e) was cleaved with HindIII and a fragment of 2 kb containing the whole ORF was excised. This fragment was inserted

into the HindIII site of a expression-plasmid YEp52 having a promoter Gal10, the expression of which was induced by galactose in a medium. The plasmid having the scaurl^s gene which had been inserted in such a direction as to be normally transcribed by the promoter Gal10 was named YEpSCARW3. Fig. 9 shows the structure of this plasmid. Further, the plasmid having the scaurl^s gene inserted in the opposite direction was named YEpSCARW1.

5-b) Transformation by plasmids YEpSCARW3 and YEpSCARW1

By using 5 μg portions of the plasmids YEpSCARW3 and YEpSCARW1, the diploid S. cerevisiae cells with the disrupted scaurl^s gene prepared in Example 3-b) were transformed. Then transformants were screened on an SD agar plate. These transformants were allowed to undergo sporulation on an SP medium and then subjected to the tetrad analysis. When the expression of the scaurl^s gene was induced by using a YPGal medium (1% of yeast extract, 2% of polypeptone, 2% of galactose), the ascospores formed from the diploid cells transformed by YEpSCARW3 all underwent germination while two of the four ascospores formed from the diploid cells transformed by YEpSCARW1 underwent germination but not the remaining two. It is thus conceivable that the cells with the disrupted scaurl^s gene have reverted to the normal state by introducing YEpSCARW3 containing the scaurl^s gene into these cells. Accordingly, the use of these cells with the

disrupted scaurl^s gene as a host makes it possible to determine the activity of normal aurl-analogous genes carried by other organisms.

Example 6: Confirmation and cloning of aurl and aur2 genes (caaurl, caaur2) carried by C. albicans

6-a) Detection of aurl gene by the PCR method

Poly(A)$^+$RNA was extracted and purified from an aureobasidin sensitive strain C. albicans TIMM0136 by the same method as the one employed in Example 4. By using the poly(A)$^+$RNA (5 μg) as a template, a double-stranded cDNA was synthesized on a cDNA synthesizing system Plus (manufactured by Amersham) with the use of an oligo(dT) primer. Mixed primers for PCR corresponding to amino acid sequence regions being common to the amino acid sequences of S. cerevisiae and Schizo. pombe were synthesized on a DNA synthesizer and purified. That is to say, a primer of SEQ ID No. 11 in Sequence Listing corresponding to the region of amino acids at the 184- to 192-positions of SEQ ID No. 4 in Sequence Listing of Schizo. pombe (from the 184- to 192-positions of SEQ ID No. 8 in Sequence Listing of S. cerevisiae) and another primer of SEQ ID No. 12 in Sequence Listing corresponding to the region of amino acids from the 289- to 298-positions of Schizo. pombe (from the 289- to 298-positions of SEQ ID No. 8 in Sequence Listing of S. cerevisiae) were employed.

PCR was performed by using these primers and the above-mentioned cDNA as a template by repeating a cycle comprising treatment at 94°C for 30 seconds, one at 48°C for 1 minute and one at 72°C for 2 minutes 25 times. As a result, a DNA (about 350 bp) being almost the same as S. cerevisiae and Schizo. pombe in length was amplified (Fig. 6). Fig. 6 shows a pattern obtained by carrying out PCR with the use of cDNA of C. albicans (lane 1), cDNA of S. cerevisiae (lane 2) and cDNA of Schizo. pombe (lane 3) as a template, electrophoresing each PCR product on an agarose gel and staining with ethidium bromide.

6-b) Cloning of aur1 gene (caaur1) of C. albicans

(i) Genomic DNA was extracted and purified from a strain C. albicans TIMM0136 by the same method as the one described in Example 1-c). After partially digesting with HindIII, the DNA fragment was ligated with a Traplex119 vector which had been completely digested with HindIII and transformed into E. coli HB101. Thus a genomic library of C. albicans was prepared. From this library, a DNA fragment of 4.5 kb containing the aur1 gene of C. albicans was cloned by using the DNA fragment of C. albicans obtained by the PCR described in Example 6-a), which had been labeled with [α-^{32}P]dCTP by using a random primer DNA labeling kit (manufactured by Takara Shuzo Co., Ltd.), as a probe. This DNA fragment had a restriction enzyme map shown in Fig. 7 and the DNA nucleotide sequence thereof is represented by SEQ ID No. 13 in Sequence

Listing. Based on this nucleotide sequence, it was estimated that the caaur1 gene coded for a protein having the amino acid sequence represented by SEQ ID No. 14 in Sequence Listing. When compared with the scaur1^{s} protein, a homology of as high as 53% was observed. A Traplex119 vector having this caaur1 gene integrated therein was named pCAAR1, while E. coli HB101 transformed by this plasmid was named and designated as Escherichia coli HB101/pCAAR1. This strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4482.

Next, pCAAR1 was treated with HindIII to thereby give caaur1 of 4.5 kb. Further, it was integrated into pTV118 which had been completely digested with HindIII to thereby prepare a plasmid for expressing caaur1. This plasmid was named pTCAAR1.

(ii) Genomic DNA was extracted and parified from a strain C. albicans TIMM1768 [The journal of Antibiotics, 46, 1414-1420(1993)] by the same method as the one described in Example 1-c). After partially digesting with Hind III, the DNA fragment was ligated with a pUC118 vector which had been completely digested with Hind III and transformed into E. coli HB101. Thus a genomic library of C. albicans TIMM1768 was prepared. From this library, a DNA fragment of 4.5 kb containing the aur1 gene of C. albicans TIMM1768 was cloned by the colony hybridization with the same

probe as that described in Example 6-b)-(i). This DNA fragment had the same restriction enzyme map as that shown in Fig. 7. Next, a part of the DNA sequence containing a ORF in this DNA fragment was determined. The DNA nucleotide sequence thereof is represented by SEQ ID No. 21 in Sequence Listing. Based on this nucleotide sequence, it was estimated that this gene coded for a protein having the amino acid sequence represented by SEQ ID No. 22 in Sequence Listing. When the amino acid sequence of the caaurl protein C. albicans TIMM1768 was compared with that of the caaurl protein of C. albicans TIMM0136, the amino acid sequences of the 1- to 381-positions and the 383- to 423-positions and the 425- to 471-positions of caaurl protein (SEQ ID No. 14 in Sequence Listing) in C. albicans TIMM0136 were identical with the amino acid sequences of the 2- to 382-positions and the 384- to 424-positions and the 426- to 472-positions, respectively, of caaurl protein (SEQ ID No. 22 in Sequence Listing) in C. albicans TIMM1768.

However, serines at the 382- and 424-positions of SEQ ID No. 14 in Sequence Listing were replaced with prolines at the 383- and 425-positions of SEQ ID No. 22 in Sequence Listing.

6-c) Cloning of aur2 gene (caaur2) of C. albicans

Genomic DNA of a strain C. albicans TIMM0136 was digested with BamHI and ligated with a pTV118 vector which had been completely digested with BamHI. Then it

was transformed into *E. coli* HB101 to thereby prepare a genomic library of *C. albicans*. On the other hand, the DNA fragment containing the scaur2^s gene obtained in Example 2-h) was cleaved with *Hin*dIII and *Pst*I to thereby give a DNA fragment of 1.2 kb. This DNA fragment was labeled with [α-^{32}P]dCTP by using a random primer DNA labeling kit. By using this labeled DNA fragment as a probe, the above-mentioned *C. albicans* genomic library was screened by the colony hybridization. Thus a plasmid containing a DNA fragment of 8.3 kb was obtained. A part of the DNA sequence upstream of the *Bam*HI site of this DNA fragment was determined (SEQ ID No. 15 in Sequence Listing). Based on this sequence, an amino acid sequence represented by SEQ ID No. 16 in Sequence Listing was estimated. It corresponded to the amino acid sequence of the 1230- to 1309-positions of the amino acid sequence of the scaur2 gene (SEQ ID No. 10), having a homology of as high as 77%. Since this DNA fragment lacked a part of the C-end, the genomic library prepared in Example 6-b) was further screened by using this DNA fragment as a probe. Thus a DNA fragment of 6.5 kb having the C-terminal part was obtained. Fig. 8 shows the restriction enzyme map of the DNA region containing the caaur2 gene thus clarified.

A pTV118 vector having the above-mentioned caaur2 gene of 8.3 kb integrated therein was named pCAAR2N, while *E. coli* HB101 transformed by this plasmid was

named and designated as *Escherichia coli* HB101/pCAAR2N. This strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4481.

Example 7: Preparation of antibody against protein coded for by scaur1^{s} gene and staining of *S. cerevisiae* cells and detection of said protein by using this antibody.

7-a) Preparation of antibody

SCAR1-1 (SEQ ID No. 19 in Sequence Listing) comprising a peptide corresponding to the amino acids at the residue 103 to 113 in the amino acid sequence of SEQ ID No. 8 in Sequence Listing having cysteine added to the N-end thereof and SCAR1-2 (SEQ ID No. 20 in Sequence Listing) comprising a peptide corresponding to the amino acids at the residue 331 to 348 in the amino acid sequence of SEQ ID No. 8 having cysteine added to the N-end thereof were synthesized by the Fmoc solid phase synthesis method and purified by reverse phase HPLC. Thus 10 mg portions of these peptides were obtained. To the N-terminal cysteine of each of these synthetic peptides, KLH was bound as a carrier protein. By using this binding product as an antigen, a rabbit was immunized and an antiserum was obtained. This antiserum was further purified on an affinity column prepared by binding the synthetic

peptide employed as the antigen to an agarose gel. This a polyclonal antibody being specific for the synthetic peptide was prepared.

7-b) Staining of S. cerevisiae cells with antibody

A strain S. cervisiae ATCC 9763 was cultured in a YNBG medium [0.67% of yeast nitrogen base (manufactured by Difco), 2% of glucose] to thereby give a suspension of a concentration of 3 x 10^7 cells/ml. To 1 ml of this cell suspension were added 0.11 ml of a 1 M phosphate buffer (pH 6.5) and 0.17 ml of 37% formaldehyde. After slowly stirring at room temperature for 1 hour, the cells were harvested by centrifugation and then suspended in 20 ml of an SS buffer (1 M of sorbitol, 0.2 % of β-mercaptoethanol, 0.1 M phosphate buffer, pH 7.5) containing 20 μg/ml of Zymolyase 20T. After treating at 30℃ for 1 hour, the cells were harvested, washed with the SS buffer, suspended in 1 ml of the SS buffer containing 0.1% of Triton X-100 and then allowed to stand for 10 minutes. This cell suspension was placed on a slide glass which had been coated with poly(L-lysine) and allowed to stand for 10 minutes. Next, a PBS solution containing 1% of albumin (BSA) was dropped thereinto. After allowing to stand at room temperature for 15 minutes, the excessive liquid was removed and then a PBS solution containing BSA containing 0.02 mg/ml of the antiSCAR1-1 antibody was dropped thereinto. After allowing to stand at room temperature for 60 minutes and washing with PBS containing BSA three times,

antirabbit IgG antibody labeled with FITC (antibody concentration 0.02 mg/ml) was layered over and allowed to stand at room temperature for 1 hour. After washing with a PBS solution containing BSA, a small amount of a mountain solution, which was a solution prepared by dissolving 0.1 g of p-phenylenediamine in 10 ml of CBS (150 mM of NaCl, 50 mM of CHES, pH 9.5), adjusting the pH value to 9.0 with 10 N NaOH and further adding 90 ml of glycerol, was layered over. Then a cover glass was placed thereon to thereby give a specimen. This specimen was observed under a fluorescence microscope to thereby examine the intracellular distribution of the scaurl protein. As a result,it was found out that this protein was distributed all over the cells.

7-c) Detection of protein coded for by scaurl gene by using antibody

The plasmid YEpSCARW3 prepared in Example 5-a) was introduced into a normal haploid S. cerevisiae SH3328 to thereby give a transformant. This transformant was cultured in a YPGal medium or a YPD medium and the cells were harvested by centrifugation. The cells thus obtained were suspended in a buffer (1% of Triton X-100, 1% of SDS, 20 mM of Tris-HCl, pH 7.9, 10 mM of EDTA, 1 mM of DTT, 1 mM of PMSF). Further, glass beads were added thereto to disrupt the cells by vigorous vortex. Then an SDS loading solution was added thereto and the protein was denatured by treating at 95°C for 5 minutes. After centrifuging, a part of the obtained supernatant was subjected to SDS-PAGE and the protein

thus separated was transfered onto an Immobilon membrane (manufactured by MILLIPORE). This Immobilon membrane was treated with Block Ace (manufactured by Dainippon Pharmaceutical Co., Ltd.). Then the antiSCAR1-2 antibody prepared in 7-a) was reacted therewith as a primary antibody. After washing, antirabbit IgG antibody labeled with peroxidase was reacted therewith as a secondary antibody and the mixture was thoroughly washed. Next, it was color-developed with diaminobenzidine and a band of the scaur1 protein was detected. Fig. 11 shows the results.

Fig. 11 shows the results of the detection of the protein prepared from the cells incubated in the YPD medium (lane 1) and the protein prepared from the cells incubated in the YPGal medium (lane 2), each subjected to SDS-PAGE, by using the antiSCAR1-2 antibody. The cells incubated in the YPGal medium, of which scaur1 gene had been induced, showed a specific band.

[Effects of the Invention]

According to the present invention, a novel protein regulating aureobasidin sensitivity and a gene coding for the protein, i.e., a gene regulating aureobasidin sensitivity are provided. These substances are useful in the diagnosis and treatment for diseases caused by organisms having the above-mentioned gene, such as mycoses. The present invention further provides an

antisense DNA and an antisense RNA of this gene, a nucleic acid probe being hybridizable with the gene, a process for detecting the gene by using this nucleic acid probe, a process for producing a protein regulating aureobasidin sensitivity by using a transformant having the gene introduced thereinto, an antibody for the protein and a process for detecting the protein by using this antibody. They are also useful in the diagnosis and treatment of diseases including mycoses.

Sequence Listing

SEQ ID NO : 1

SEQUENCE LENGTH : 2385

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

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TAATAAACTA ATTATATTAT ATATAATTAG CAATTTTATA AAAAAAATAA AAAAATAGCC 120
CTGATTGCTG GCAACTGTGA GCTGAACATT GGTTAATCGG TCCATCTTTT TTTAAATATT 180
TTACATCGCT ACTTTTAAGT GCTTGACACT TGCATTTAAT AGCTACTTTC TTTCCTTCAT 240
AAAAATTCCT TTTTTTTCCT TTAGTTTTCC GGTTAATTCC TTACGAAATT TTTTTCGTAC 300
GCTTCCCTTT TTTACTCTGA TAATTCTTTG AAGCAATGTC TGCTCTTTCG ACCTTAAAAA 360
AGCGCCTTGC TGCGTGTAAC CGAGCATCCC AATACAAGTT GGAAACAAGC TTAAACCCTA 420
TGCCTACATT TCGTTTGCTA CGCAATACGA AATGGTCATG GACACATTTG CAATATGTCT 480
TTCTAGCAGG TAATTTGATT TTTGCTTGTA TTGTCATTGA ATCTCCTGGA TTCTGGGGCA 540
AATTTGGCAT TGCCTGTCTT TTGGCCATTG CGTTGACCGT TCCTTTAACA CGCCAAATTT 600
TTTTTCCTGC CATTGTTATC ATCACCTGGG CAATTTTATT TTACTCTTGT AGGTTTATTC 660
CAGAACGCTG GCGTCCACCC ATATGGGTTC GTGTTTTACC CACACTTGAA AATATTCTTT 720
ATGGCTCTAA TCTTTCTAGT CTTCTCTCGA AAACCACGCA TAGCATCCTT GATATTTTGG 780
CCTGGGTTCG ATATGGAGTC ATGCATTATT CGGCTCCTTT TATCATTTCA TTTATTCTTT 840
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ATGGTTTAGA ACCTGCCACG TATGCAGTAC GTGGCTCTCC TGGTGGATTG GCCCGTATTG 1020
ATGCTCTCTT CGGCACTAGC ATTTACACTG ATTGTTTTTC TAACTCTCCG GTTGTTTTTG 1080
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TGTACCTTAC CCACCACTAC TTTGTAGATT TGGTCGGCGG TATGTGTTTA GCTATTATAT 1260

GCTTCGTTTT TGCTCAAAAG CTACGCCTCC CACAGTTGCA AACTGGTAAA ATCCTTCGTT 1320

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CTCGTACCGG CAGCCCATAC TTACTTGGAA GGGATTCTTT TACTCAAAAC CCTAATGCAG 1440

TAGCCTTCAT GAGTGGTCTT AACAATATGG AACTTGCTAA CACCGATCAT GAATGGTCCG 1500

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GAAATTAAGC AGCTTTGACT AACTACATTT TTGTTTTTTT CCTAAGCAAA ACTGTATAGT 2340

TATACACGCG AGCTGTATTC ACTTCCATTG TAGTGACTTG AGCTC 2385

SEQ ID NO : 2

SEQUENCE LENGTH : 422

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

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Arg Ala Ser Gln Tyr Lys Leu Glu Thr Ser Leu Asn Pro Met Pro

20 25 30

Thr Phe Arg Leu Leu Arg Asn Thr Lys Trp Ser Trp Thr His Leu

35 40 45

Gln Tyr Val Phe Leu Ala Gly Asn Leu Ile Phe Ala Cys Ile Val

50 55 60

Ile Glu Ser Pro Gly Phe Trp Gly Lys Phe Gly Ile Ala Cys Leu

65 70 75

Leu Ala Ile Ala Leu Thr Val Pro Leu Thr Arg Gln Ile Phe Phe

80 85 90

Pro Ala Ile Val Ile Ile Thr Trp Ala Ile Leu Phe Tyr Ser Cys

95 100 105

Arg Phe Ile Pro Glu Arg Trp Arg Pro Pro Ile Trp Val Arg Val

110 115 120

Leu Pro Thr Leu Glu Asn Ile Leu Tyr Gly Ser Asn Leu Ser Ser

125 130 135

Leu Leu Ser Lys Thr Thr His Ser Ile Leu Asp Ile Leu Ala Trp

140 145 150

Val Pro Tyr Gly Val Met His Tyr Ser Ala Pro Phe Ile Ile Ser

155 160 165

Phe Ile Leu Phe Ile Phe Ala Pro Pro Gly Thr Leu Pro Val Trp

170 175 180

Ala Arg Thr Phe Gly Tyr Met Asn Leu Phe Gly Val Leu Ile Gln

185 190 195

Met Ala Phe Pro Cys Ser Pro Pro Trp Tyr Glu Asn Met Tyr Gly

200 205 210

Leu Glu Pro Ala Thr Tyr Ala Val Arg Gly Ser Pro Gly Gly Leu

215 220 225

Ala Arg Ile Asp Ala Leu Phe Gly Thr Ser Ile Tyr Thr Asp Cys

230 235 240

Phe Ser Asn Ser Pro Val Val Phe Gly Ala Phe Pro Ser Leu His

245 250 255

Ala Gly Trp Ala Met Leu Glu Ala Leu Phe Leu Ser His Val Phe

260 265 270

Pro Arg Tyr Arg Phe Cys Phe Tyr Gly Tyr Val Leu Trp Leu Cys

275 280 285

Trp Cys Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Val

290 295 300

Gly Gly Met Cys Leu Ala Ile Ile Cys Phe Val Phe Ala Gln Lys

305 310 315

Leu Arg Leu Pro Gln Leu Gln Thr Gly Lys Ile Leu Arg Trp Glu

320 325 330

Tyr Glu Phe Val Ile His Gly His Gly Leu Ser Glu Lys Thr Ser

335 340 345

Asn Ser Leu Ala Arg Thr Gly Ser Pro Tyr Leu Leu Gly Arg Asp

350 355 360

Ser Phe Thr Gln Asn Pro Asn Ala Val Ala Phe Met Ser Gly Leu

365 370 375

Asn Asn Met Glu Leu Ala Asn Thr Asp His Glu Trp Ser Val Gly

380 385 390

Ser Ser Ser Pro Glu Pro Leu Pro Ser Pro Ala Ala Asp Leu Ile

395 400 405

Asp Arg Pro Ala Ser Thr Thr Ser Ser Ile Phe Asp Ala Ser His

410 415 420

Leu Pro

SEQ ID NO : 3

SEQUENCE LENGTH : 2385

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

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TTCTAGCAGG TAATTTGATT TTTGCTTGTA TTGTCATTGA ATCTCCTGGA TTCTGGGGGA  540
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TATCCAGCTT CTTACACTGT CAACTGTTGT GAAAGGAACA CATTTAGAAT TTCATTTTCC 1980

TTATTTGTTG TGATTTAAAT CGTTTGACAT AATTTTAAAT TTGGTTTGAA ATGTGTGTGA 2040

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GAAATTAAGC AGCTTTGACT AACTACATTT TTGTTTTTTT CCTAAGCAAA ACTGTATAGT 2340

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SEQ ID NO : 4

SEQUENCE LENGTH : 422

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Ser Ala Leu Ser Thr Leu Lys Lys Arg Leu Ala Ala Cys Asn
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Arg Ala Ser Gln Tyr Lys Leu Glu Thr Ser Leu Asn Pro Met Pro
                20   25   30

Thr Phe Arg Leu Leu Arg Asn Thr Lys Trp Ser Trp Thr His Leu

35 40 45

Gln Tyr Val Phe Leu Ala Gly Asn Leu Ile Phe Ala Cys Ile Val

50 55 60

Ile Glu Ser Pro Gly Phe Trp Gly Lys Phe Gly Ile Ala Cys Leu

65 70 75

Leu Ala Ile Ala Leu Thr Val Pro Leu Thr Arg Gln Ile Phe Phe

80 85 90

Pro Ala Ile Val Ile Ile Thr Trp Ala Ile Leu Phe Tyr Ser Cys

95 100 105

Arg Phe Ile Pro Glu Arg Trp Arg Pro Pro Ile Trp Val Arg Val

110 115 120

Leu Pro Thr Leu Glu Asn Ile Leu Tyr Gly Ser Asn Leu Ser Ser

125 130 135

Leu Leu Ser Lys Thr Thr His Ser Ile Leu Asp Ile Leu Ala Trp

140 145 150

Val Pro Tyr Gly Val Met His Tyr Ser Ala Pro Phe Ile Ile Ser

155 160 165

Phe Ile Leu Phe Ile Phe Ala Pro Pro Gly Thr Leu Pro Val Trp

170 175 180

Ala Arg Thr Phe Gly Tyr Met Asn Leu Phe Gly Val Leu Ile Gln

185 190 195

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Phe Ser Asn Ser Pro Val Val Phe Gly Ala Phe Pro Ser Leu His

245 250 255

Ala Gly Trp Ala Met Leu Glu Ala Leu Phe Leu Ser His Val Phe

260 265 270

Pro Arg Tyr Arg Phe Cys Phe Tyr Gly Tyr Val Leu Trp Leu Cys

275 280 285

Trp Cys Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Val

290 295 300

Gly Gly Met Cys Leu Ala Ile Ile Cys Phe Val Phe Ala Gln Lys

305 310 315

Leu Arg Leu Pro Gln Leu Gln Thr Gly Lys Ile Leu Arg Trp Glu

320 325 330

Tyr Glu Phe Val Ile His Gly His Gly Leu Ser Glu Lys Thr Ser

335 340 345

Asn Ser Leu Ala Arg Thr Gly Ser Pro Tyr Leu Leu Gly Arg Asp

350 355 360

Ser Phe Thr Gln Asn Pro Asn Ala Val Ala Phe Met Ser Gly Leu

365 370 375

Asn Asn Met Glu Leu Ala Asn Thr Asp His Glu Trp Ser Val Gly

380 385 390

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Asp Arg Pro Ala Ser Thr Thr Ser Ser Ile Phe Asp Ala Ser His

410 415 420

Leu Pro

SEQ ID NO : 5

SEQUENCE LENGTH : 2340

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

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TTTCTTTCTG TCAAAGAATA ATAAAGTGCC CATCAGTGTT CATATTTGTT ACAAAGTGGT   60
TTTCTGATTT GGTACTACTG CAGAGGCGTA TTTTTTGCTT CAGTTACCAT AGCGTAAGAA  120
CACTAGCGAC TTTTGTTCGT GAACCAACAG AGTAGGATTT CTACTGCTAC ATCTCTTAGG  180
TAGTTGGTTA GTCCGATCGC TCACTTTTGG TTGTTGTTAA GTACTTCATA AGTTTATCCT  240
TTTCCTTTTT CACACTGAGC TACTTTGGGT ATAGCTTTTG GCCCAAGGAT CTTTGAATTT  300
TCTCCAAAAG TACTTTATTT TATATCCTAC AGGTTGCGGT TTTCATATTT TAAAAAGCTT  360
TTTAATCATT CCTTTGCGTA TGGCAAACCC TTTTTCGAGA TGGTTTCTAT CAGAGAGACC  420
TCCAAACTGC CATGTAGCCG ATTTAGAAAC AAGTTTAGAT CCCCATCAAA CGTTGTTGAA  480
GGTGCAAAAA TACAAACCCG CTTTAAGCGA CTGGGTGCAT TACATCTTCT TGGGATCCAT  540
CATGCTGTTT GTGTTCATTA CTAATCCCGC ACCTTGGATC TTCAAGATCC TTTTTTATTG  600
TTTCTTGGGC ACTTTATTCA TCATTCCAGC TACGTCACAG TTTTTCTTCA ATGCCTTGCC  660
CATCCTAACA TGGGTGGCGC TGTATTTCAC TTCATCGTAC TTTCCAGATG ACCGCAGGCC  720
TCCTATTACT GTCAAAGTGT TACCAGCGGT GGAAACAATT TTATACGGCG ACAATTTAAG  780
TGATATTCTT GCAACATCGA CGAATTCCTT TTTGGACATT TTAGCATGGT TACCGTACGG  840
ACTATTTCAT TATGGGGCCC CATTTGTCGT TGCTGCCATC TTATTCGTAT TTGGTCCACC  900
AACTGTTTTG CAAGGTTATG CTTTTGCATT TGGTTATATG AACCTGTTTG GTGTTATCAT  960
GCAAAATGTC TTTCCAGCCG CTCCCCCATG GTATAAAATT CTCTATGGAT TGCAATCAGC 1020
CAAGTATGAT ATGCATGGCT CGCCTGGTGG ATTAGCTAGA ATTGATAAGC TACTCGGTAT 1080
TAATATGTAT ACTACAGCTT TTTCAAATTC CTCCGTCATT TTCGGTGCTT TTCCTTCACT 1140
GCATTCCGGG TGTGCTACTA TGGAAGCCCT GTTTTTCTGT TATTGTTTTC CAAAATTGAA 1200
GCCCTTGTTT ATTGCTTATG TTTGCTGGTT ATGGTGGTCA ACTATGTATC TGACACACCA 1260
TTATTTTGTA GACCTTATGG CAGGTTCTGT GCTGTCATAC GTTATTTTCC AGTACACAAA 1320
GTACACACAT TTACCAATTG TAGATACATC TCTTTTTTGC AGATGGTCAT ACACTTCAAT 1380
TGAGAAATAC GATATATCAA AGAGTGATCC ATTGGCTGCA GATTCAAACG ATATCGAAAG 1440
TGTCCCTTTG TCCAACTTGG AACTTGACTT TGATCTTAAT ATGACTGATG AACCCAGTGT 1500
AAGCCCTTCG TTATTTGATG GATCTACTTC TGTTTCTCGT TCGTCCGCCA CGTCTATAAC 1560
GTCACTAGGT GTAAAGAGGG CTTAATGAGT ATTTTATCTG CAATTACGGA TACGGTTGGT 1620
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CTTATGTAGA TACATATAAA TATATATCTT TTTCTTTCTT TTTCTTAGTC AGGATTGTCG 1680

TTTAGCATAA TATACATGTA GTTTATTTAA TCACATACCA CTGATTATCT TTAGAATTTT 1740

ATAAATTTTT GAAATAAATG GGTGGCTTTT AATGGTGTCT ATGTTAAGTG AGGCTTTTAG 1800

AATGCTCTTC CTGCTTTGTT TATTATATGT GTATGAAAGA TATGTATGTA TTTACATGTG 1860

TTTGTAGCGT CCCCAGTCAA AACCTGTGCG CTATACCTAA ATGGATTGAT AATCTTCATT 1920

CACTAATTCT AAAATAGACT TCTTCCCCAA AGAACGGTGT AACGATGAGG CTCTATCCAG 1980

CTGCTTATCT AAATCAACTT TAACGATGGA TGATCTTATG ACACGGGGAT CTTTCTTTAA 2040

AGTTCTTAGA ATTTCAGACT GTACCGCAGC TGATGAATCA AACAGCATTA AAAAGTGATA 2100

TGCTCGAAAA TGTTTTTCCT GGTCTTTCTT CATTATTTTA GGAAGATACC TTATGCCCAT 2160

GGGTACAATG TCCCTCACCA CACCTCTGTT TTGAATAATC AGTTTCCCGA TTGTGGAAGA 2220

CAATTCTTTT GCTTCCAACT TTGGCGCATT GGAGTTGGTT ATGCGAACAA GTCCGATCAG 2280

CTCATAAAGC ATCTTAGTGA AAAGGGTGGT TTTGCGTTAT TCTTTCCTCT GTTGAAGCTT 2340

SEQ ID NO : 6

SEQUENCE LENGTH : 401

SEQUENCE TYPE : amino acid

STRANDED : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Ala Asn Pro Phe Ser Arg Trp Phe Leu Ser Glu Arg Pro Pro
1 5 10 15

Asn Cys His Val Ala Asp Leu Glu Thr Ser Leu Asp Pro His Gln
20 25 30

Thr Leu Leu Lys Val Gln Lys Tyr Lys Pro Ala Leu Ser Asp Trp
35 40 45

Val His Tyr Ile Phe Leu Gly Ser Ile Met Leu Phe Val Phe Ile
50 55 60

Thr Asn Pro Ala Pro Trp Ile Phe Lys Ile Leu Phe Tyr Cys Phe

65 70 75

Leu Gly Thr Leu Phe Ile Ile Pro Ala Thr Ser Gln Phe Phe Phe

80 85 90

Asn Ala Leu Pro Ile Leu Thr Trp Val Ala Leu Tyr Phe Thr Ser

95 100 105

Ser Tyr Phe Pro Asp Asp Arg Arg Pro Pro Ile Thr Val Lys Val

110 115 120

Leu Pro Ala Val Glu Thr Ile Leu Tyr Gly Asp Asn Leu Ser Asp

125 130 135

Ile Leu Ala Thr Ser Thr Asn Ser Phe Leu Asp Ile Leu Ala Trp

140 145 150

Leu Pro Tyr Gly Leu Phe His Tyr Gly Ala Pro Phe Val Val Ala

155 160 165

Ala Ile Leu Phe Val Phe Gly Pro Pro Thr Val Leu Gln Gly Tyr

170 175 180

Ala Phe Ala Phe Gly Tyr Met Asn Leu Phe Gly Val Ile Met Gln

185 190 195

Asn Val Phe Pro Ala Ala Pro Pro Trp Tyr Lys Ile Leu Tyr Gly

200 205 210

Leu Gln Ser Ala Asn Tyr Asp Met His Gly Ser Pro Gly Gly Leu

215 220 225

Ala Arg Ile Asp Lys Leu Leu Gly Ile Asn Met Tyr Thr Thr Ala

230 235 240

Phe Ser Asn Ser Ser Val Ile Phe Gly Ala Phe Pro Ser Leu His

245 250 255

Ser Gly Cys Ala Thr Met Glu Ala Leu Phe Phe Cys Tyr Cys Phe

260 265 270

Pro Lys Leu Lys Pro Leu Phe Ile Ala Tyr Val Cys Trp Leu Trp

275 280 285

Trp Ser Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Met

290 295 300

Ala Gly Ser Val Leu Ser Tyr Val Ile Phe Gln Tyr Thr Lys Tyr

305 310 315

Thr His Leu Pro Ile Val Asp Thr Ser Leu Phe Cys Arg Trp Ser

320 325 330

Tyr Thr Ser Ile Glu Lys Tyr Asp Ile Ser Lys Ser Asp Pro Leu

335 340 345

Ala Ala Asp Ser Asn Asp Ile Glu Ser Val Pro Leu Ser Asn Leu

350 355 360

Glu Leu Asp Phe Asp Leu Asn Met Thr Asp Glu Pro Ser Val Ser

365 370 375

Pro Ser Leu Phe Asp Gly Ser Thr Ser Val Ser Arg Ser Ser Ala

380 385 390

Thr Ser Ile Thr Ser Leu Gly Val Lys Arg Ala

395 400

SEQ ID NO : 7

SEQUENCE LENGTH : 2340

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

TTTCTTTCTG TCAAAGAATA ATAAAGTGCC CATCAGTGTT CATATTTGTT ACAAAGTGGT 60

TTTCTGATTT GGTACTACTG CAGAGGCGTA TTTTTTGCTT CAGTTACCAT AGCGTAAGAA 120

CACTAGCGAC TTTTGTTCGT GAACCAACAG AGTAGGATTT CTACTGCTAC ATCTCTTAGG 180

TAGTTGGTTA GTCCGATCGC TCACTTTTGG TTGTTGTTAA GTACTTCATA AGTTTATCCT 240

TTTCCTTTTT CACACTGAGC TACTTTGGGT ATAGCTTTTG GCCCAAGGAT CTTTGAATTT 300

TCTCCAAAAG TACTTTATTT TATATCCTAC AGGTTGCGGT TTTCATATTT TAAAAAGCTT 360

TTTAATCATT CCTTTGCGTA TGGCAAACCC TTTTTCGAGA TGGTTTCTAT CAGAGAGACC 420

TCCAAACTGC CATGTAGCCG ATTTAGAAAC AAGTTTAGAT CCCCATCAAA CGTTGTTGAA 480

GGTGCAAAAA TACAAACCCG CTTTAAGCGA CTGGGTGCAT TACATCTTCT TGGGATCCAT 540

CATGCTGTTT GTGTTCATTA CTAATCCCGC ACCTTGGATC TTCAAGATCC TTTTTTATTG 600

TTTCTTGGGC ACTTTATTCA TCATTCCAGC TACGTCACAG TTTTTCTTCA ATGCCTTGCC 660

CATCCTAACA TGGGTGGCGC TGTATTTCAC TTCATCGTAC TTTCCAGATG ACCGCAGGCC 720

TCCTATTACT GTCAAAGTGT TACCAGCGGT GGAAACAATT TTATACGGCG ACAATTTAAG 780

TGATATTCTT GCAACATCGA CGAATTCCTT TTTGGACATT TTAGCATGGT TACCGTACGG 840

ACTATTTCAT TTTGGGGCCC CATTTGTCGT TGCTGCCATC TTATTCGTAT TTGGTCCACC 900

AACTGTTTTG CAAGGTTATG CTTTTGCATT TGGTTATATG AACCTGTTTG GTGTTATCAT 960

GCAAAATGTC TTTCCAGCCG CTCCCCCATG GTATAAAATT CTCTATGGAT TGCAATCAGC 1020

CAACTATGAT ATGCATGGCT CGCCTGGTGG ATTAGCTAGA ATTGATAAGC TACTCGGTAT 1080

TAATATGTAT ACTACAGCTT TTTCAAATTC CTCCGTCATT TTCGGTGCTT TTCCTTCACT 1140

GCATTCCGGG TGTGCTACTA TGGAAGCCCT GTTTTTCTGT TATTGTTTTC CAAAATTGAA 1200

GCCCTTGTTT ATTGCTTATG TTTGCTGGTT ATGGTGGTCA ACTATGTATC TGACACACCA 1260

TTATTTTGTA GACCTTATGG CAGGTTCTGT GCTGTCATAC GTTATTTTCC AGTACACAAA 1320

GTACACACAT TTACCAATTG TAGATACATC TCTTTTTTGC AGATGGTCAT ACACTTGAAT 1380

TGAGAAATAC GATATATCAA AGAGTGATCC ATTGGCTGCA GATTCAAACG ATATCGAAAG 1440

TGTCCCTTTG TCCAACTTGG AACTTGACTT TGATCTTAAT ATGACTGATG AACCCAGTGT 1500

AAGCCCTTCG TTATTTGATG GATCTACTTC TGTTTCTCGT TCGTCCGCCA CGTCTATAAC 1560

GTCACTAGGT GTAAAGAGGG CTTAATGAGT ATTTTATCTG CAATTACGGA TACGGTTGGT 1620

CTTATGTAGA TACATATAAA TATATATCTT TTTCTTTCTT TTTCTTAGTC AGGATTGTCG 1680

TTTAGCATAA TATACATGTA GTTTATTTAA TCACATACCA CTGATTATCT TTAGAATTTT 1740

ATAAATTTTT GAAATAAATG GGTGGCTTTT AATGGTGTCT ATGTTAAGTG AGGCTTTTAG 1800

AATGCTCTTC CTGCTTTGTT TATTATATGT GTATGAAAGA TATGTATGTA TTTACATGTG 1860

TTTGTAGCGT CCCCAGTCAA AACCTGTGCG CTATACCTAA ATGGATTGAT AATCTTCATT 1920

CACTAATTCT AAAATAGACT TCTTCCCCAA AGAACGGTGT AACGATGAGG CTCTATCCAG 1980

CTGCTTATCT AAATCAACTT TAACGATGGA TGATCTTATG ACACGGGGAT CTTTCTTTAA 2040

AGTTCTTAGA ATTTCAGACT GTACCGCAGC TGATGAATCA AACAGCATTA AAAAGTGATA 2100

TGCTCGAAAA TGTTTTTCCT GGTCTTTCTT CATTATTTTA GGAAGATACC TTATGCCCAT 2160

GGGTACAATG TCCCTCACCA CACCTCTGTT TTGAATAATC AGTTTCCCGA TTGTGGAAGA 2220

CAATTCTTTT GCTTCCAACT TTGGCGCATT GGAGTTGGTT ATGCGAACAA GTCCGATCAG 2280

CTCATAAAGC ATCTTAGTGA AAAGGGTGGT TTTGCGTTAT TCTTTCCTCT GTTGAAGCTT 2340

SEQ ID NO : 8

SEQUENCE LENGTH : 401

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Ala Asn Pro Phe Ser Arg Trp Phe Leu Ser Glu Arg Pro Pro
1 5 10 15

Asn Cys His Val Ala Asp Leu Glu Thr Ser Leu Asp Pro His Gln
20 25 30

Thr Leu Leu Lys Val Gln Lys Tyr Lys Pro Ala Leu Ser Asp Trp
35 40 45

Val His Tyr Ile Phe Leu Gly Ser Ile Met Leu Phe Val Phe Ile
50 55 60

Thr Asn Pro Ala Pro Trp Ile Phe Lys Ile Leu Phe Tyr Cys Phe
65 70 75

Leu Gly Thr Leu Phe Ile Ile Pro Ala Thr Ser Gln Phe Phe Phe
80 85 90

Asn Ala Leu Pro Ile Leu Thr Trp Val Ala Leu Tyr Phe Thr Ser
95 100 105

Ser Tyr Phe Pro Asp Asp Arg Arg Pro Pro Ile Thr Val Lys Val
110 115 120

Leu Pro Ala Val Glu Thr Ile Leu Tyr Gly Asp Asn Leu Ser Asp

125 130 135

Ile Leu Ala Thr Ser Thr Asn Ser Phe Leu Asp Ile Leu Ala Trp

140 145 150

Leu Pro Tyr Gly Leu Phe His Phe Gly Ala Pro Phe Val Val Ala

155 160 165

Ala Ile Leu Phe Val Phe Gly Pro Pro Thr Val Leu Gln Gly Tyr

170 175 180

Ala Phe Ala Phe Gly Tyr Met Asn Leu Phe Gly Val Ile Met Gln

185 190 195

Asn Val Phe Pro Ala Ala Pro Pro Trp Tyr Lys Ile Leu Tyr Gly

200 205 210

Leu Gln Ser Ala Asn Tyr Asp Met His Gly Ser Pro Gly Gly Leu

215 220 225

Ala Arg Ile Asp Lys Leu Leu Gly Ile Asn Met Tyr Thr Thr Ala

230 235 240

Phe Ser Asn Ser Ser Val Ile Phe Gly Ala Phe Pro Ser Leu His

245 250 255

Ser Gly Cys Ala Thr Met Glu Ala Leu Phe Phe Cys Tyr Cys Phe

260 265 270

Pro Lys Leu Lys Pro Leu Phe Ile Ala Tyr Val Cys Trp Leu Trp

275 280 285

Trp Ser Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Met

290 295 300

Ala Gly Ser Val Leu Ser Tyr Val Ile Phe Gln Tyr Thr Lys Tyr

305 310 315

Thr His Leu Pro Ile Val Asp Thr Ser Leu Phe Cys Arg Trp Ser

320 325 330

Tyr Thr Ser Ile Glu Lys Tyr Asp Ile Ser Lys Ser Asp Pro Leu

335 340 345

Ala Ala Asp Ser Asn Asp Ile Glu Ser Val Pro Leu Ser Asn Leu

350 355 360

Glu Leu Asp Phe Asp Leu Asn Met Thr Asp Glu Pro Ser Val Ser

365 370 375

Pro Ser Leu Phe Asp Gly Ser Thr Ser Val Ser Arg Ser Ser Ala

380 385 390

Thr Ser Ile Thr Ser Leu Gly Val Lys Arg Ala

395 400

SEQ ID NO : 9

SEQUENCE LENGTH : 5340

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

```
AGCGCTTCTA TTTTCCTCCC CACCGCGAGG CGGAAATGGC ACATTTTTTT TCTTTTGCTT   60
CTGTGCTTTT GCTGTAATTT TTGGCATGTG CTATTGTATG AAGATAACGC GTGGTTCCGT  120
GGAAATAGCC GGAAATTTTG CCGGGAATAT GACGGACATG ATTTAACACC CGTGGAAATG  180
AAAAAAGCCA AGGTAAGAAA GTGGCAATAT TTTTCCTACA AATAGATCTG CTGTCCCTTA  240
GATGATTACC ATACATATAT ATATTTATTA CACACATCTG TCAGAGGTAG CTAGCGAAGG  300
TGTCACTGAA ATATTTTTTG TTCCAGTTAG TATAAATACG GAGGTAGAAC AGCTCTCCGC  360
GTGTATATCT TTTTTTGCGC TATACAAGAA CAGGAAGAAC GCATTTCCAT ACCTTTTTCT  420
CCTTACAGGT GCCCTCTGAG TAGTGTCACG AACGAGGAAA AAGATTAATA TTACTGTTTT  480
TATATTCAAA AAGAGTAAAG CCGTTGCTAT ATACGAATAT GACGATTACC GTGGGGGATG  540
CAGTTTCGGA GACGGAGCTG GAAAACAAAA GTCAAAACGT GGTACTATCT CCCAAGGCAT  600
CTGCTTCTTC AGACATAAGC ACAGATGTTG ATAAAGACAC ATCGTCTTCT TGGGATGACA  660
AATCTTTGCT GCCTACAGGT GAATATATTG TGGACAGAAA TAAGCCCCAA ACCTACTTGA  720
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ATAGCGATGA TATCGAAAAA GTGACAGAAT CTGATATTTT CCCTCAGAAA CGTCTGTTTT 780

CATTCTTGCA CTCTAAGAAA ATTCCAGAAG TACCACAAAC CGATGACGAG AGGAAGATAT 840

ATCCTCTGTT CCATACAAAT ATTATCTCTA ACATGTTTTT TTGGTGGGTT CTACCCATCC 900

TGCGAGTTGG TTATAAGAGA ACGATACAGC CGAACGATCT CTTCAAAATG GATCCGAGGA 960

TGTCTATAGA GACCCTTTAT GACGACTTTG AAAAAAACAT GATTTACTAT TTTGAGAAGA 1020

CGAGGAAAAA ATACCGTAAA AGACATCCAG AAGCGACAGA AGAAGAGGTT ATGGAAAATG 1080

CCAAACTACC TAAACATACA GTTCTGAGAG CTTTATTATT CACTTTTAAG AAACAGTACT 1140

TCATGTCGAT AGTGTTTGCA ATTCTCGCTA ATTGTACATC CGGTTTTAAC CCCATGATTA 1200

CCAAGAGGCT AATTGAGTTT GTCGAAGAAA AGGCTATTTT TCATAGCATG CATGTTAACA 1260

AAGGTATTGG TTACGCTATT GGTGCATGTT TGATGATGTT CGTTAACGGG TTGACGTTCA 1320

ATCATTTCTT TCATACATCC CAACTGACTG GTGTGCAAGC TAAGTCTATT CTTACTAAAG 1380

CTGCCATGAA GAAAATGTTT AATGCATCTA ATTATGCGAG ACATTGTTTT GCTAACGGTA 1440

AAGTGACTTC TTTTGTAACA ACAGATCTCG CTAGAATTGA ATTTGCCTTA TCTTTTCAGC 1500

CGTTTTTGGC TGGGTTCCCT GCAATTTTGG CTATTTGCAT TGTTTTATTG ATCGTTAACC 1560

TTGGACCCAT TGCCTTAGTT GGGATTGGTA TTTTTTTCGG TGGGTTTTTC ATATCCTTAT 1620

TTGCATTTAA GTTAATTCTG GGCTTTAGAA TTGCTGCGAA CATCTTCACT GATGCTAGAG 1680

TTACCATGAT GAGAGAAGTG CTGAATAATA TAAAAATGAT TAAATATTAT ACGTGGGAGG 1740

ATGCGTATGA AAAAAATATT CAAGATATTA GGACCAAAGA GATTTCTAAA GTTAGAAAAA 1800

TGCAACTATC AAGAAATTTC TTGATTGCTA TGGCCATGTC TTTGCCTAGT ATTGCTTCAT 1860

TGGTCACTTT CCTTGCAATG TACAAAGTTA ATAAAGGAGG CAGGCAACCT GGTAATATTT 1920

TTGCCTCTTT ATCTTTATTT CAGGTCTTGA GTTTGCAAAT GTTTTTCTTA CCTATTGCTA 1980

TTGGTACTGG AATTGACATG ATCATTGGAT TGGGCCGTTT GCAAAGCTTA TTGGAGGCTC 2040

CAGAAGATGA TCCAAATCAG ATGATTGAAA TGAAGCCCTC TCCTGGCTTT GATCCAAAAT 2100

TGGCTCTAAA AATGACACAT TGCTCATTTG AGTGGGAAGA TTATGAATTA AACGACGCTA 2160

TTGAAGAAGC AAAAGGAGAA GCTAAAGATG AAGGTAAAAA GAACAAAAAA AAGCGTAAGG 2220

ATACATGGGG TAAGCCATCT GCAAGTACTA ATAAGGCGAA AAGATTGGAC AATATGTTGA 2280

AAGACAGAGA CGGCCCGGAA GATTTAGAAA AAACTTCGTT TAGGGGTTTC AAGGACTTGA 2340

ACTTCGATAT TAAAAAGGGC GAATTTATTA TGATTACGGG ACCTATTGGT ACTGGTAAAT 2400

CTTCATTATT GAATGCGATG GCAGGATCAA TGAGAAAAAT TGATGGTAAG GTTGAAGTCA 2460

ACGGGGACTT ATTAATGTGT GGTTATCCAT GGATTCAAAA TGCATCTGTA AGAGATAACA 2520
TCATATTCGG TTCACCATTC AATAAAGAAA AGTATGATGA AGTAGTTCGT GTTTGCTCTT 2580
TGAAAGCTGA TCTGGATATT TTACCGGCAG GCGATATGAC CGAAATTGGG GAACGTGGTA 2640
TTACTTTATC TGGTGGTCAA AAGGCACGTA TCAATTTAGC CAGGTCTGTT TATAAGAAGA 2700
AGGATATTTA TGTATTCGAC GATGTCCTAA GTGCTGTCGA TTCTCGTGTT GGTAAACACA 2760
TCATGGATGA ATGTCTAACC GGAATGCTTG CTAATAAAAC CAGAATTTTA GCAACGCATC 2820
AGTTGTCACT GATTGAGAGA GCTTCTAGAG TCATCGTTTT AGGTACTGAT GGCCAAGTCG 2880
ATATTGGTAC TGTTGATGAG CTAAAAGCTC GTAATCAAAC TTTGATAAAT CTTTTACAAT 2940
TCTCTTCTCA AAATTCGGAG AAAGAGGATG AAGAACAGGA AGCGGTTGTT TCCGGTGAAT 3000
TGGGACAACT AAAATATGAA CCAGAGGTAA AGGAATTGAC TGAACTGAAG AAAAAGGCTA 3060
CAGAAATGTC ACAAACTGCA AATAGTGGTA AAATTGTAGC GGATGGTCAT ACTAGTAGTA 3120
AAGAAGAAAG AGCAGTCAAT AGTATCAGTC TGAAAATATA CCGTGAATAC ATTAAAGCTG 3180
CAGTAGGTAA GTGGGGTTTT ATCGCACTAC CGTTGTATGC AATTTTAGTC GTTGGAACCA 3240
CATTCTGCTC ACTTTTTTCT TCCGTTTGGT TATCTTACTG GACTGAGAAT AAATTCAAAA 3300
ACAGACCACC CAGTTTTTAT ATGGGTCTTT ACTCCTTCTT TGTGTTTGCT GCTTTCATAT 3360
TCATGAATGG CCAGTTCACC ATACTTTGCG CAATGGGTAT TATGGCATCG AAATGGTTAA 3420
ATTTGAGGGC TGTGAAAAGA ATTTTACACA CTCCAATGTC ATACATAGAT ACCACACCTT 3480
TGGGACGTAT TCTGAACAGA TTCACAAAAG ATACAGATAG CTTAGATAAT GAGTTAACCG 3540
AAAGTTTACG GTTGATGACA TCTCAATTTG CTAATATTGT AGGTGTTTGC GTCATGTGTA 3600
TTGTTTACTT GCCGTGGTTT GCTATCGCAA TTCCGTTTCT TTTGGTCATC TTTGTTCTGA 3660
TTGCTGATCA TTATCAGAGT TCTGGTAGAG AAATTAAAAG ACTTGAAGCT GTGCAACGGT 3720
CTTTTGTTTA CAATAATTTA AATGAAGTTT TGGGTGGGAT GGATACAATC AAAGCATACC 3780
GAAGTCAGGA ACGATTTTTG GCGAAATCAG ATTTTTTGAT CAACAAGATG AATGAGGCGG 3840
GATACCTTGT AGTTGTCCTG CAAAGATGGG TAGGTATTTT CCTTGATATG GTTGCTATCG 3900
CATTTGCACT AATTATTACG TTATTGTGTG TTACGAGAGC CTTTCCTATT TCCGCGGCTT 3960
CAGTTGGTGT TTTGTTGACT TATGTATTAC AATTGCCTGG TCTATTAAAT ACCATTTTAA 4020
GGGCAATGAC TCAAACAGAG AATGACATGA ATAGTGCCGA AAGATTGGTA ACATATGCAA 4080
CTGAACTACC ACTAGAGGCA TCCTATAGAA AGCCCGAAAT GACACCTCCA GAGTCATGGC 4140
CCTCAATGGG CGAAATAATT TTTGAAAATG TTGATTTTGC CTATAGACCT GGTTTACCTA 4200

TAGTTTTAAA AAATCTTAAC TTGAATATCA AGAGTGGGGA AAAAATTGGT ATCTGTGGTC 4260

GTACAGGTGC TGGTAAGTCC ACTATTATGA GTGCCCTTTA CAGGTTGAAT GAATTGACCG 4320

CAGGTAAAAT TTTAATTGAC AATGTTGATA TAAGTCAGCT GGGACTTTTC GATTTAAGAA 4380

GAAAATTAGC CATCATTCCA CAAGATCCAG TATTATTTAG GGGTACGATT CGCAAGAACT 4440

TAGATCCATT TAATGAGCGT ACAGATGACG AATTATGGGA TGCATTGGTG AGAGGTGGTG 4500

CTATCGCCAA GGATGACTTG CCGGAAGTGA AATTGCAAAA ACCTGATGAA AATGGTACTC 4560

ATGGTAAAAT GCATAAGTTC CATTTAGATC AAGCAGTGGA AGAAGAGGGC TCCAATTTCT 4620

CCTTAGGTGA GAGACAACTA TTAGCATTAA CAAGGGCATT GGTCCGCCAA TCAAAAATAT 4680

TGATTTTGGA TGAGGCTACA TCCTCAGTGG ACTACGAAAC GGATGGCAAA ATCCAAACAC 4740

GTATTGTTGA GGAATTTGGA GATTGTACAA TTTTGTGTAT TGCTCACAGA CTGAAGACCA 4800

TTGTAAATTA TGATCGTATT CTTGTTTTAG AGAAGGGTGA AGTCGCAGAA TTCGATACAC 4860

CATGGACGTT GTTTAGTCAA GAAGATAGTA TTTTCAGAAG CATGTGTTCT AGATCTGGTA 4920

TTGTGGAAAA TGATTTCGAG AACACAAGTT AATTTATATT ATTTGTTGCA TGATTTTTCT 4980

CTTTTATTTA TTTATATCTT GCCGATGGTA CAAATTAGTA CTAGAAAAGA AAACCCACTA 5040

CTATGACTTG CAGAAAAAGT TATGTGTGCC ATAGATAGAT ATAATTGCAT ACCCACATCG 5100

TATACTCAAA ATTCCGAAAA GAACATTTCA TTTTTTATGA GGCAAACTGA ACAACGCTTC 5160

GGTCCTTTTT TCATTCTAGA AATATATATT TATACATCAT TTTCAGAAGA TATTCAAAGA 5220

ACTTATTGGG ATGTCTATTT ACTGAATAAA GTATACACAA AAAACGAATT TAAAATGGAA 5280

GGCATAAATA GAAAACTTAG AAGTGAAAAT CCTAAAACCG AAGGATATTT CAAATACGTA 5340

SEQ ID NO : 10

SEQUENCE LENGTH : 1477

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Thr Ile Thr Val Gly Asp Ala Val Ser Glu Thr Glu Leu Glu

5 10 15

Asn Lys Ser Gln Asn Val Val Leu Ser Pro Lys Ala Ser Ala Ser

20 25 30

Ser Asp Ile Ser Thr Asp Val Asp Lys Asp Thr Ser Ser Ser Trp

35 40 45

Asp Asp Lys Ser Leu Leu Pro Thr Gly Glu Tyr Ile Val Asp Arg

50 55 60

Asn Lys Pro Gln Thr Tyr Leu Asn Ser Asp Asp Ile Glu Lys Val

65 70 75

Thr Glu Ser Asp Ile Phe Pro Gln Lys Arg Leu Phe Ser Phe Leu

80 85 90

His Ser Lys Lys Ile Pro Glu Val Pro Gln Thr Asp Asp Glu Arg

95 100 105

Lys Ile Tyr Pro Leu Phe His Thr Asn Ile Ile Ser Asn Met Phe

110 115 120

Phe Trp Trp Val Leu Pro Ile Leu Arg Val Gly Tyr Lys Arg Thr

125 130 135

Ile Gln Pro Asn Asp Leu Phe Lys Met Asp Pro Arg Met Ser Ile

140 145 150

Glu Thr Leu Tyr Asp Asp Phe Glu Lys Asn Met Ile Tyr Tyr Phe

155 160 165

Glu Lys Thr Arg Lys Lys Tyr Arg Lys Arg His Pro Glu Ala Thr

170 175 180

Glu Glu Glu Val Met Glu Asn Ala Lys Leu Pro Lys His Thr Val

185 190 195

Leu Arg Ala Leu Leu Phe Thr Phe Lys Lys Gln Tyr Phe Met Ser

200 205 210

Ile Val Phe Ala Ile Leu Ala Asn Cys Thr Ser Gly Phe Asn Pro

215 220 225

Met Ile Thr Lys Arg Leu Ile Glu Phe Val Glu Glu Lys Ala Ile

230 235 240

Phe His Ser Met His Val Asn Lys Gly Ile Gly Tyr Ala Ile Gly

245 250 255

Ala Cys Leu Met Met Phe Val Asn Gly Leu Thr Phe Asn His Phe

260 265 270

Phe His Thr Ser Gln Leu Thr Gly Val Gln Ala Lys Ser Ile Leu

275 280 285

Thr Lys Ala Ala Met Lys Lys Met Phe Asn Ala Ser Asn Tyr Ala

290 295 300

Arg His Cys Phe Pro Asn Gly Lys Val Thr Ser Phe Val Thr Thr

305 310 315

Asp Leu Ala Arg Ile Glu Phe Ala Leu Ser Phe Gln Pro Phe Leu

320 325 330

Ala Gly Phe Pro Ala Ile Leu Ala Ile Cys Ile Val Leu Leu Ile

335 340 345

Val Asn Leu Gly Pro Ile Ala Leu Val Gly Ile Gly Ile Phe Phe

350 355 360

Gly Gly Phe Phe Ile Ser Leu Phe Ala Phe Lys Leu Ile Leu Gly

365 370 375

Phe Arg Ile Ala Ala Asn Ile Phe Thr Asp Ala Arg Val Thr Met

380 385 390

Met Arg Glu Val Leu Asn Asn Ile Lys Met Ile Lys Tyr Tyr Thr

395 400 405

Trp Glu Asp Ala Tyr Glu Lys Asn Ile Gln Asp Ile Arg Thr Lys

410 415 420

Glu Ile Ser Lys Val Arg Lys Met Gln Leu Ser Arg Asn Phe Leu

425 430 435

Ile Ala Met Ala Met Ser Leu Pro Ser Ile Ala Ser Leu Val Thr

440 445 450

Phe Leu Ala Met Tyr Lys Val Asn Lys Gly Gly Arg Gln Pro Gly

455 460 465

Asn Ile Phe Ala Ser Leu Ser Leu Phe Gln Val Leu Ser Leu Gln

470 475 480

Met Phe Phe Leu Pro Ile Ala Ile Gly Thr Gly Ile Asp Met Ile

485 490 495

Ile Gly Leu Gly Arg Leu Gln Ser Leu Leu Glu Ala Pro Glu Asp

500 505 510

Asp Pro Asn Gln Met Ile Glu Met Lys Pro Ser Pro Gly Phe Asp

515 520 525

Pro Lys Leu Ala Leu Lys Met Thr His Cys Ser Phe Glu Trp Glu

530 535 540

Asp Tyr Glu Leu Asn Asp Ala Ile Glu Glu Ala Lys Gly Glu Ala

545 550 555

Lys Asp Glu Gly Lys Lys Asn Lys Lys Lys Arg Lys Asp Thr Trp

560 565 570

Gly Lys Pro Ser Ala Ser Thr Asn Lys Ala Lys Arg Leu Asp Asn

575 580 585

Met Leu Lys Asp Arg Asp Gly Pro Glu Asp Leu Glu Lys Thr Ser

590 595 600

Phe Arg Gly Phe Lys Asp Leu Asn Phe Asp Ile Lys Lys Gly Glu

605 610 615

Phe Ile Met Ile Thr Gly Pro Ile Gly Thr Gly Lys Ser Ser Leu

620 625 630

Leu Asn Ala Met Ala Gly Ser Met Arg Lys Ile Asp Gly Lys Val

635 640 645

Glu Val Asn Gly Asp Leu Leu Met Cys Gly Tyr Pro Trp Ile Gln

650 655 660

Asn Ala Ser Val Arg Asp Asn Ile Ile Phe Gly Ser Pro Phe Asn

665 670 675

Lys Glu Lys Tyr Asp Glu Val Val Arg Val Cys Ser Leu Lys Ala

680 685 690

Asp Leu Asp Ile Leu Pro Ala Gly Asp Met Thr Glu Ile Gly Glu

695 700 705

Arg Gly Ile Thr Leu Ser Gly Gly Gln Lys Ala Arg Ile Asn Leu

710 715 720

Ala Arg Ser Val Tyr Lys Lys Lys Asp Ile Tyr Val Phe Asp Asp

725 730 735

Val Leu Ser Ala Val Asp Ser Arg Val Gly Lys His Ile Met Asp

740 745 750

Glu Cys Leu Thr Gly Met Leu Ala Asn Lys Thr Arg Ile Leu Ala

755 760 765

Thr His Gln Leu Ser Leu Ile Glu Arg Ala Ser Arg Val Ile Val

770 775 780

Leu Gly Thr Asp Gly Gln Val Asp Ile Gly Thr Val Asp Glu Leu

785 790 795

Lys Ala Arg Asn Gln Thr Leu Ile Asn Leu Leu Gln Phe Ser Ser

800 805 810

Gln Asn Ser Glu Lys Glu Asp Glu Glu Gln Glu Ala Val Val Ser

815 820 825

Gly Glu Leu Gly Gln Leu Lys Tyr Glu Pro Glu Val Lys Glu Leu

830 835 840

Thr Glu Leu Lys Lys Lys Ala Thr Glu Met Ser Gln Thr Ala Asn

845 850 855

Ser Gly Lys Ile Val Ala Asp Gly His Thr Ser Ser Lys Glu Glu

860 865 870

Arg Ala Val Asn Ser Ile Ser Leu Lys Ile Tyr Arg Glu Tyr Ile

875 880 885

Lys Ala Ala Val Gly Lys Trp Gly Phe Ile Ala Leu Pro Leu Tyr

890 895 900

Ala Ile Leu Val Val Gly Thr Thr Phe Cys Ser Leu Phe Ser Ser

905 910 915

Val Trp Leu Ser Tyr Trp Thr Glu Asn Lys Phe Lys Asn Arg Pro

920 925 930

Pro Ser Phe Tyr Met Gly Leu Tyr Ser Phe Phe Val Phe Ala Ala

935 940 945

Phe Ile Phe Met Asn Gly Gln Phe Thr Ile Leu Cys Ala Met Gly

950 955 960

Ile Met Ala Ser Lys Trp Leu Asn Leu Arg Ala Val Lys Arg Ile

965 970 975

Leu His Thr Pro Met Ser Tyr Ile Asp Thr Thr Pro Leu Gly Arg

980 985 990

Ile Leu Asn Arg Phe Thr Lys Asp Thr Asp Ser Leu Asp Asn Glu

995 1000 1005

Leu Thr Glu Ser Leu Arg Leu Met Thr Ser Gln Phe Ala Asn Ile

1010 1015 1020

Val Gly Val Cys Val Met Cys Ile Val Tyr Leu Pro Trp Phe Ala

1025 1030 1035

Ile Ala Ile Pro Phe Leu Leu Val Ile Phe Val Leu Ile Ala Asp

1040 1045 1050

His Tyr Gln Ser Ser Gly Arg Glu Ile Lys Arg Leu Glu Ala Val

1055 1060 1065

Gln Arg Ser Phe Val Tyr Asn Asn Leu Asn Glu Val Leu Gly Gly

1070 1075 1080

Met Asp Thr Ile Lys Ala Tyr Arg Ser Gln Glu Arg Phe Leu Ala

1085 1090 1095

Lys Ser Asp Phe Leu Ile Asn Lys Met Asn Glu Ala Gly Tyr Leu

1100 1105 1110

Val Val Val Leu Gln Arg Trp Val Gly Ile Phe Leu Asp Met Val

1115 1120 1125

Ala Ile Ala Phe Ala Leu Ile Ile Thr Leu Leu Cys Val Thr Arg

1130 1135 1140

Ala Phe Pro Ile Ser Ala Ala Ser Val Gly Val Leu Leu Thr Tyr

1145 1150 1155

Val Leu Gln Leu Pro Gly Leu Leu Asn Thr Ile Leu Arg Ala Met

1160 1165 1170

Thr Gln Thr Glu Asn Asp Met Asn Ser Ala Glu Arg Leu Val Thr

1175 1180 1185

Tyr Ala Thr Glu Leu Pro Leu Glu Ala Ser Tyr Arg Lys Pro Glu

1190 1195 1200

Met Thr Pro Pro Glu Ser Trp Pro Ser Met Gly Glu Ile Ile Phe

1205 1210 1215

Glu Asn Val Asp Phe Ala Tyr Arg Pro Gly Leu Pro Ile Val Leu

1220 1225 1230

Lys Asn Leu Asn Leu Asn Ile Lys Ser Gly Glu Lys Ile Gly Ile

1235 1240 1245

Cys Gly Arg Thr Gly Ala Gly Lys Ser Thr Ile Met Ser Ala Leu

1250 1255 1260

Tyr Arg Leu Asn Glu Leu Thr Ala Gly Lys Ile Leu Ile Asp Asn

1265 1270 1275

Val Asp Ile Ser Gln Leu Gly Leu Phe Asp Leu Arg Arg Lys Leu

1280 1285 1290

Ala Ile Ile Pro Gln Asp Pro Val Leu Phe Arg Gly Thr Ile Arg

1295 1300 1305

Lys Asn Leu Asp Pro Phe Asn Glu Arg Thr Asp Asp Glu Leu Trp

1310 1315 1320

Asp Ala Leu Val Arg Gly Gly Ala Ile Ala Lys Asp Asp Leu Pro
1325 1330 1335
Glu Val Lys Leu Gln Lys Pro Asp Glu Asn Gly Thr His Gly Lys
1340 1345 1350
Met His Lys Phe His Leu Asp Gln Ala Val Glu Glu Glu Gly Ser
1355 1360 1365
Asn Phe Ser Leu Gly Glu Arg Gln Leu Leu Ala Leu Thr Arg Ala
1370 1375 1380
Leu Val Arg Gln Ser Lys Ile Leu Ile Leu Asp Glu Ala Thr Ser
1385 1390 1395
Ser Val Asp Tyr Glu Thr Asp Gly Lys Ile Gln Thr Arg Ile Val
1400 1405 1410
Glu Glu Phe Gly Asp Cys Thr Ile Leu Cys Ile Ala His Arg Leu
1415 1420 1425
Lys Thr Ile Val Asn Tyr Asp Arg Ile Leu Val Leu Glu Lys Gly
1430 1435 1440
Glu Val Ala Glu Phe Asp Thr Pro Trp Thr Leu Phe Ser Gln Glu
1445 1450 1455
Asp Ser Ile Phe Arg Ser Met Cys Ser Arg Ser Gly Ile Val Glu
1460 1465 1470
Asn Asp Phe Glu Asn Arg Ser
1475

SEQ ID NO : 11

SEQUENCE LENGTH : 26

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : Other nucleic acid(synthetic DNA)

SEQUENCE DESCRIPTION :

TTTGGTTAYA TGAAYYTNTT YGGNGT 26

SEQ ID NO : 12

SEQUENCE LENGTH : 29

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : Other nucleic acid(synthetic DNA)

SEQUENCE DESCRIPTION :

TCTACAAART ARTGGTGNGT NARRTACAT 29

SEQ ID NO : 13

SEQUENCE LENGTH : 2274

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

TTATATATAT TATTGATTTG TTCCTGTTGT TATTTAGTTT AGAATCAGAC GACTACACCA 60
GAACCACAAT TCAACCAACA CTTATATAGA ACCTGGCTTG GAAAAAAGTA ACATTTATCA 120
TTCCTATACT TTTTTAGCAA ACATAATCCG TGTTTTACAT ATATTATTCA CCCAATATCA 180
TAACAAAAAC AAACTGAATA ATGGCGTCTT CTATTTTGCG TTCCAAAATA ATACAAAAAC 240
CGTACCAATT ATTCCACTAC TATTTTCTTC TGGAGAAGGC TCCTGGTTCT ACAGTTAGTG 300
ATTTGAATTT TGATACAAAC ATACAAACGA GTTTACGTAA ATTAAAGCAT CATCATTGGA 360
CGGTGGGAGA AATATTCCAT TATGGGTTTT TGGTTTCCAT ACTTTTTTTC GTGTTTGTGG 420
TTTTCCCAGC TTCATTTTTT ATAAAATTAC CAATAATCTT AGCATTTGCT ACTTGTTTTT 480
TAATACCCTT AACATCACAA TTTTTTCTTC CTGCCTTGCC CGTTTTCACT TGGTTGGCAT 540
TATATTTTAC GTGTGCTAAA ATACCTCAAG AATGGAAACC AGCTATCACA GTTAAAGTTT 600
TACCAGCTAT GGAAACAATT TTGTACGGCG ATAATTTATC AAATGTTTTG GCAACCATCA 660

CTACCGGAGT GTTAGATATA TTGGCATGGT TACCATATGG GATTATTCAT TTCAGTTTCC 720
CATTTGTACT TGCTGCTATT ATATTTTTAT TTGGGCCACC GACGGCATTA AGATCATTTG 780
GATTTGCCTT TGGTTATATG AACTTGCTTG GAGTCTTGAT TCAAATGGCA TTCCCAGCTG 840
CTCCTCCATG GTACAAAAAC TTGCACGGAT TAGAACCAGC TAATTATTCA ATGCACGGGT 900
CTCCTGGTGG ACTTGGAAGG ATAGATAAAT TGTTAGGTGT TGATATGTAT ACCACAGGGT 960
TTTCCAATTC ATCAATCATT TTTGGGGCAT TCCCATCGTT ACATTCAGGA TGTTGTATCA 1020
TGGAAGTGTT ATTTTTGTGT TGGTTGTTTC CACGATTCAA GTTTGTGTGG GTTACATACG 1080
CATCTTGGCT TTGGTGGAGC ACGATGTATT TGACCCATCA CTACTTTGTC GATTTGATTG 1140
GTGGAGCCAT GCTATCTTTG ACTGTTTTTG AGTTCACCAA ATATAAATAT TTGCCAAAAA 1200
ACAAAGAAGG CCTTTTCTGT CGTTGGTCAT ACACTGAAAT TGAAAAAATC GATATCCAAG 1260
AGATTGACCC TTTATCATAC AATTATATCC CTGTCAACAG CAATGATAAT GAAAGCAGAT 1320
TGTATACGAG AGTGTACCAA GAGTCTCAGG TTAGTCCCCC ACAGAGAGCT GAAACACCTG 1380
AAGCATTTGA GATGTCAAAT TTTTCTAGGT CTAGACAAAG CTCAAAGACT CAGGTTCCAT 1440
TGAGTAATCT TACTAACAAT GATCAAGTGT CTGGAATTAA CGAAGAGGAT GAAGAAGAAG 1500
AAGGCCGATGA AATTTCATCG AGTACTCCTT CGGTGTTTGA AGACGAACCA CAGGGTAGCA 1560
CATATGCTGC ATCCTCAGCT ACATCAGTAG ATGATTTGGA TTCCAAAAGA AATTAGTAAA 1620
ATAACAGTTT CTATTAATTT CTTTATTTCC TCCTAATTAA TGATTTTATG CTCAATACCT 1680
ACACTATCTG TTTTTAATTT CCTACTTTTT TTTTATTATT GTTGAGTTCA TTTGCTGTTC 1740
ATTGAATATT TACAATTTTG GATTAATTAC CATCAATATA GAATGGGCAC AGTTTTTTTA 1800
AGTTTTTTTG TTTTTGTGTT TGTCTTTCTT TTTTTACATT AATGTGTTTG GATTGTTTTA 1860
GGTTCCTTTA TCCCTTAGCC CCCTCAGAAT ACTATTTTAT CTAATTAATT TGTTTTTATT 1920
TTCTGATATT TACCAATTGC TTTTTCTTTT GGATATTTAT AATAGCATCC CCTAATAATT 1980
AATATACAAC TGTTTCATAT ATATACGTGT ATGTCCTGTA GTGGTGGAAA CTGGAGTCAA 2040
CATTTGTATT AATGTGTACA AGAAAGCAGT GTTAATGCTA CTATTATAAT TTTTGAGGTG 2100
CAAATCAAGA GGTTGGCAGC TTTCTTATGG CTATGACCGT GAATGAAGGC TTGTAAACCA 2160
CGTAATAAAC AAAAGCCAAC AAGTTTTTTT AGAGCCTTTA ACAACATACG CAATGAGAGT 2220
GATTGCAATA CTACAAGATA TAGCCCAAAA AATTGAATGC ATTTCAACAA CAAC 2274

SEQ ID NO : 14

SEQUENCE LENGTH : 471

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

```
Met Ala Ser Ser Ile Leu Arg Ser Lys Ile Ile Gln Lys Pro Tyr
                5                   10                  15
Gln Leu Phe His Tyr Tyr Phe Leu Ser Glu Lys Ala Pro Gly Ser
                20                  25                  30
Thr Val Ser Asp Leu Asn Phe Asp Thr Asn Ile Gln Thr Ser Leu
                35                  40                  45
Arg Lys Leu Lys His His His Trp Thr Val Gly Glu Ile Phe His
                50                  55                  60
Tyr Gly Phe Leu Val Ser Ile Leu Phe Phe Val Phe Val Val Phe
                65                  70                  75
Pro Ala Ser Phe Phe Ile Lys Leu Pro Ile Ile Leu Ala Phe Ala
                80                  85                  90
Thr Cys Phe Leu Ile Pro Leu Thr Ser Gln Phe Phe Leu Pro Ala
                95                  100                 105
Leu Pro Val Phe Thr Trp Leu Ala Leu Tyr Phe Thr Cys Ala Lys
                110                 115                 120
Ile Pro Gln Glu Trp Lys Pro Ala Ile Thr Val Lys Val Leu Pro
                125                 130                 135
Ala Met Glu Thr Ile Leu Tyr Gly Asp Asn Leu Ser Asn Val Leu
                140                 145                 150
Ala Thr Ile Thr Thr Gly Val Leu Asp Ile Leu Ala Trp Leu Pro
                155                 160                 165
Tyr Gly Ile Ile His Phe Ser Phe Pro Phe Val Leu Ala Ala Ile
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170 175 180

Ile Phe Leu Phe Gly Pro Pro Thr Ala Leu Arg Ser Phe Gly Phe

185 190 195

Ala Phe Gly Tyr Met Asn Leu Leu Gly Val Leu Ile Gln Met Ala

200 205 210

Phe Pro Ala Ala Pro Pro Trp Tyr Lys Asn Leu His Gly Leu Glu

215 220 225

Pro Ala Asn Tyr Ser Met His Gly Ser Pro Gly Gly Leu Gly Arg

230 235 240

Ile Asp Lys Leu Leu Gly Val Asp Met Tyr Thr Thr Gly Phe Ser

245 250 255

Asn Ser Ser Ile Ile Phe Gly Ala Phe Pro Ser Leu His Ser Gly

260 265 270

Cys Cys Ile Met Glu Val Leu Phe Leu Cys Trp Leu Phe Pro Arg

275 280 285

Phe Lys Phe Val Trp Val Thr Tyr Ala Ser Trp Leu Trp Trp Ser

290 295 300

Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Ile Gly Gly

305 310 315

Ala Met Leu Ser Leu Thr Val Phe Glu Phe Thr Lys Tyr Lys Tyr

320 325 330

Leu Pro Lys Asn Lys Glu Gly Leu Phe Cys Arg Trp Ser Tyr Thr

335 340 345

Glu Ile Glu Lys Ile Asp Ile Gln Glu Ile Asp Pro Leu Ser Tyr

350 355 360

Asn Tyr Ile Pro Val Asn Ser Asn Asp Asn Glu Ser Arg Leu Tyr

365 370 375

Thr Arg Val Tyr Gln Glu Ser Gln Val Ser Pro Pro Gln Arg Ala

380 385 390

Glu Thr Pro Glu Ala Phe Glu Met Ser Asn Phe Ser Arg Ser Arg
395 400 405
Gln Ser Ser Lys Thr Gln Val Pro Leu Ser Asn Leu Thr Asn Asn
410 415 420
Asp Gln Val Ser Gly Ile Asn Glu Glu Asp Glu Glu Glu Glu Gly
425 430 435
Asp Glu Ile Ser Ser Ser Thr Pro Ser Val Phe Glu Asp Glu Pro
440 445 450
Gln Gly Ser Thr Tyr Ala Ala Ser Ser Ala Thr Ser Val Asp Asp
455 460 465
Leu Asp Ser Lys Arg Asn
470

SEQ ID NO : 15

SEQUENCE LENGTH : 243

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

TTTGAAAAAT TTGAATTTTA AAATTAATCC AATGGAAAAA ATTGGTATTT GTGGAAGAAC 60
CGGTGCTGGT AAATCATCAA TTATGACAGC ATTATATCGA TTATCAGAAT TAGAACTGGG 120
GAAAATTATT ATTGATGATA TTGATATTTC AACTTTGGGT TTAAAAGATC TTCGATCAAA 180
ATTATCAATT ATTCCTCAAG ATCCAGTATT ATTCCGAGGT TCAATTCGGA AAAACTTGGA 240
TCC 243

SEQ ID NO : 16

SEQUENCE LENGTH : 80

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Leu Lys Asn Leu Asn Phe Lys Ile Asn Pro Met Glu Lys Ile Gly
5 10 15

Ile Cys Gly Arg Thr Gly Ala Gly Lys Ser Ser Ile Met Thr Ala
20 25 30

Leu Tyr Arg Leu Ser Glu Leu Glu Leu Gly Lys Ile Ile Ile Asp
35 40 45

Asp Ile Asp Ile Ser Thr Leu Gly Leu Lys Asp Leu Arg Ser Lys
50 55 60

Leu Ser Ile Ile Pro Gln Asp Pro Val Leu Phe Arg Gly Ser Ile
65 70 75

Arg Lys Asn Leu Asp
80

SEQ ID NO : 17

SEQUENCE LENGTH : 1601

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

ANTI-SENSE : Yes

SEQUENCE DESCRIPTION :

AGGAAGATGA CTTGCATCAA AGATGGAGGA AGTGGTACTG GCAGGACGAT CAATCAAATC 60

AGCAGCAGGA CTAGGTAACG GCTCAGGTGA TGATGAACCC ACGGACCATT CATGATCGGT 120

GTTAGCAAGT TCCATATTGT TAAGACCACT CATGAAGGCT ACTGCATTAG GGTTTTGAGT 180

AAAAGAATCC CTTCCAAGTA AGTATGGGCT GCCGGTACGA GCCAAGGAGT TGCTGGTTTT 240

TTCGGAAAGA CCATGACCGT GGATAACAAA CTCGTATTCC CAACGAAGGA TTTTACCAGT 300

TTGCAACTGT GGGAGGCGTA GCTTTTGAGC AAAAACGAAG CATATAATAG CTAAACACAT 360

ACCGCCGACC AAATCTACAA AGTAGTGGTG GGTAAGGTAC ATAGTACACC AGCAAAGCCA 420
TAGAACATAT CCATAAAAGC AGAAGCGGTA TCGAGGAAAC ACATGCGAAA GGAAAAGTGC 480
TTCCAGCATG GCCCATCCAG CGTGAAGAGA TGGAAAGGCA CCAAAAACAA CCGGAGAGTT 540
AGAAAAACCA TCAGTGTAAA TGCTAGTGCC GAAGAGAGCA TCAATACGGG CCAATCCACC 600
AGGAGAGCCA CGTACTGCAT ACGTGGCAGG TTCTAAACCA TACATATTTT CATACCAAGG 660
AGGAGAACAG GGGAAAGCCA TTTGGATAAG AACACCAAAT AAATTCATAT AACCAAAAGT 720
TCGAGCCCAA ACTGGAAGAG TTCCAGGAGG TGCAAAGATG AAAAGAATAA ATGAAATGAT 780
AAAAGGAGCC GAATAATGCA TGACTCCATA TGGAACCCAG GCCAAAATAT CAAGGATGCT 840
ATCCGTGGTT TTCGAGAGAA GACTAGAAAG ATTAGAGCCA TAAAGAATAT TTTCAAGTGT 900
GGGTAAAACA CGAACCCATA TGGGTGGACG CCAGCGTTCT GGAATAAACC TACAAGAGTA 960
AAATAAAATT GCCCAGGTGA TGATAACAAT GGCAGGAAAA AAAATTTGGC GTGTTAAAGG 1020
AACGGTCAAC GCAATGGCCA AAAGACAGGC AATGCCAAAT TTCCCCCAGA ATCCAGGAGA 1080
TTCAATGACA ATACAAGCAA AAATCAAATT ACCTGCTAGA AACACATATT GCAAATGTGT 1140
CCATGACCAT TTCGTATTGC GTAGCAAACG AAATGTAGGC ATAGGGTTTA AGCTTGTTTC 1200
CAACTTGTAT TGGGATGCTC GGTTACACGC AGCAAGGCGC TTTTTTAAGG TCGAAAGAGC 1260
AGACATTGCT TCAAAGAATT ATCAGAGTAA AAAAGGGAAG CGTACGAAAA AAATTTCGTA 1320
AGGAATTAAC CGGAAAACTA AAGGAAAAAA AAGGAATTTT TATGAAGGAA AGAAAGTAGC 1380
TATTAAATGC AAGTGTCAAG CACTTAAAAG TAGCGATGTA AAATATTTAA AAAAAGATGG 1440
ACCGATTAAC CAATGTTCAG CTCACAGTTG CCAGCAATCA GGGCTATTTT TTTATTTTTT 1500
TTATAAAATT GCTAATTATA TATAATATAA TTAGTTTATT AACTTGCTTT TCCTCAAAAA 1560
ACCAATTCGA GAAAGGAACT TTTGCAGAGG CAAAAAAGCT T 1601

SEQ ID NO : 18

SEQUENCE LENGTH : 1601

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : mRNA

ANTI-SENSE : Yes

SEQUENCE DESCRIPTION :

AGGAAGAUGA CUUGCAUCAA AGAUGGAGGA AGUGGUACUG GCAGGACGAU CAAUCAAAUC 60
AGCAGCAGGA CUAGGUAACG GCUCAGGUGA UGAUGAACCC ACGCACCAUU CAUGAUCGGU 120
GUUAGCAAGU UCCAUAUUCU UAAGACCACU CAUGAAGGCU ACUGCAUUAG GGUUUUGAGU 180
AAAAGAAUCC CUUCCAAGUA AGUAUGGGCU GCCGGUACGA GCCAAGGAGU UGCUGGUUUU 240
UUCGGAAAGA CCAUGACCGU GGAUAACAAA CUCGUAUUCC CAACGAAGGA UUUUACCAGU 300
UUGCAACUGU GGCAGGCGUA GCUUUUGAGC AAAAACGAAG CAUAUAAUAG CUAAACACAU 360
ACCGCCGACC AAAUCUACAA AGUAGUGGUG GGUAAGGUAC AUAGUACACC AGCAAAGCCA 420
UAGAACAUAU CCAUAAAAGC AGAAGCGGUA UCGAGGAAAC ACAUGCGAAA [illegible]AAAAGUGC 480
UUCCAGCAUG GCCCAUCCAG CGUGAAGAGA UGGAAAGGCA CCAA[illegible]ACAA CCGGAGAGUU 540
AGAAAAACCA UCAGUGUAAA UGCUAGUGCC GAAGAGAGCA UCAAUACGG[illegible] CCAAUCCACC 600
ACGAGACCCA CGUACUGCAU ACGUGGCAGG UUCUAAACCA UACAUAUUUU CAUACCAAGG 660
AGGAGAACAG GGGAAAGCCA UUUGGAUAAG AACACCAAAU AAAUUCAUAU AACCAAAAGU 720
UCGAGCCCAA ACUGGAAGAG UUCCAGGAGG UGCAAAGAUG AAAAGAAUAA AUGAAAUGAU 780
AAAAGGAGCC GAAUAAUGCA UGACUCCAUA UGGAACCCAG GCCAAAAUAU CAAGGAUGCU 840
AUGCGUGGUU UUCGAGAGAA GACUAGAAAG AUUAGAGCCA UAAAGAAUAU UUUCAAGUGU 900
GGGUAAAACA CGAACCCAUA UGGGUGGACG CCAGCGUUCU GGAAUAAACC UACAAGAGUA 960
AAAUAAAAUU GCCCAGGUGA UGAUAACAAU GGCAGGAAAA AAAAUUUGGC GUGUUAAAGG 1020
AACGGUCAAC GCAAUGGCCA AAAGACAGGC AAUGCCAAAU UUCCCCCAGA AUCCAGGAGA 1080
UUCAAUGACA AUACAAGCAA AAAUCAAAUU ACCUGCUAGA AACACAUAUU GCAAAUGUGU 1140
CCAUGACCAU UUCGUAUUGC GUAGCAAACG AAAUGUAGGC AUAGGGUUUA AGCUUGUUUC 1200
CAACUUGUAU UGGGAUGCUC GGUUACACGC AGCAAGGCGC UUUUUUAACG UCGAAAGAGC 1260
AGACAUUGCU UCAAAGAAUU AUCAGAGUAA AAAAGGGAAG CCUACGAAAA AAAUUUCCGUA 1320
AGGAAUUAAC CGGAAAACUA AAGGAAAAAA AAGGAAUUUU UAUGAAGGAA AGAAAGUAGC 1380
UAUUAAAUGC AAGUGUCAAG CACUUAAAAG UAGCGAUGUA AAAUAUUUAA AAAAAGAUGG 1440
ACCGAUUAAC CAAUGUUCAG CUCACAGUUG CCAGCAAUCA GGGCUAUUUU UUUAUUUUUU 1500
UUAUAAAAUU GCUAAUUAUA UAUAAUAUAA UUAGUUUAUU AACUUGCUUU UCCUCAAAAA 1560
ACCAAUUCGA GAAAGGAACU UUUGCAGAGG CAAAAAAGCU U 1601

SEQ ID NO : 19

SEQUENCE LENGTH : 12

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Cys Phe Thr Ser Ser Tyr Phe Pro Asp Asp Arg Arg
                    5   10

SEQ ID NO : 20

SEQUENCE LENGTH : 19

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Cys Tyr Thr Ser Ile Glu Lys Tyr Asp Ile Ser Lys Ser Asp Pro
                    5   10   15
Leu Ala Ala Asp

SEQ ID NO : 21

SEQUENCE LENGTH : 1553

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : Genomic DNA

SEQUENCE DESCRIPTION :

TTTTACATAT ATTATTCACC CAATATCATA ACAAAAACAA ACTGAATGAT GGCATCTTCT 60

ATTTTGCGTT CCAAAATAAT ACAAAAACCG TACCAATTAT TCCACTACTA TTTTCTTCTG 120

GAGAAGGCTC CTGGTTCTAC AGTTACTGAT TTGAATTTTG ATACAAACAT ACAAACGAGT 180
TTACGTAAAT TAAAGCATCA TCATTGGACG GTGGGAGAAA TATTCCATTA TGGGTTTTTG 240
GTTTCCATAC TTTTTTTCGT GTTTGTGCTT TTCCCAGCTT CATTTTTTAT AAAATTACCA 300
ATAATCTTAG CATTTGCTAC TTGTTTTTTA ATACCCTTAA CATCACAATT TTTTCTTCCT 360
GCCTTGCCCG TTTTCACTTG GTTGGCATTA TATTTTACGT GTCCTAAAAT ACCTCAAGAA 420
TGGAAACCAG CTATCACAGT TAAAGTTTTA CCAGCTATGG AAACAATTTT GTACGGCGAT 480
AATTTATCAA ATGTTTTGGC AACCATCACT ACCGGAGTGT TAGATATATT GGCATGGTTA 540
CCATATGGGA TTATTCATTT CAGTTTCCCA TTTGTACTTG CTGCTATTAT ATTTTTATTT 600
GGGCCACCGA CGGCATTAAG ATCATTTGGA TTTGCCTTTG GTTATATGAA CTTGCTTGGA 660
GTCTTGATTC AAATGGCATT CCCAGCTGCT CCTCCATGGT ACAAAAACTT GCACGGATTA 720
GAACCAGCTA ATTATTCAAT GCACGGGTCT CCTGGTGGAC TTGGAAGGAT AGATAAATTG 780
TTAGGTGTTG ATATGTATAC CACAGGGTTT TCCAATTCAT CAATCATTTT TGGGGCATTC 840
CCATCGTTAC ATTCAGGATG TTGTATCATG GAAGTGTTAT TTTTGTGTTG GTTGTTTCCA 900
CGATTCAAGT TTGTGTGGGT TACATACGCA TCTTGGCTTT GGTGGAGCAC GATGTATTTG 960
ACCCATCACT ACTTTGTCGA TTTGATTGGT GGAGCCATGC TATCTTTGAC TGTTTTTGAA 1020
TTCACCAAAT ATAAATATTT GCCAAAAAAC AAAGAAGGCC TTTTCTGTCG TTGGTCATAC 1080
ACTGAAATTG AAAAAATCGA TATCCAAGAG ATTGACCCTT TATCATACAA TTATATCCCT 1140
GTCAACAGCA ATGATAATGA AAGCAGATTG TATACGAGAG TGTACCAAGA GCCTCAGGTT 1200
AGTCCCCCAC AGAGAGCTGA AACACCTGAA GCATTTGAGA TGTCAAATTT TTCTAGGTCT 1260
AGACAAAGCT CAAAGACTCA GGTTCCATTG AGTAATCTTA CTAACAATGA TCAAGTGCCT 1320
GGAATTAACG AAGAGGATGA AGAAGAAGAA GGCGATGAAA TTTCGTCGAG TACTCCTTCG 1380
GTGTTTGAAG ACCGAACCACA GGGTAGCACA TATGCTGCAT CCTCAGCTAC ATCAGTAGAT 1440
GATTTGGATT CCAAAAGAAA TTAGTAAAAC AGCAGTTTCT ATTAATTTCT TTATTTCCTC 1500
CTAATTAATG ATTTTATGTT CAATACCTAC ACTATCTGTT TTTAATTTCC TAC 1553

SEQ ID NO : 22

SEQUENCE LENGTH : 472

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Met Ala Ser Ser Ile Leu Arg Ser Lys Ile Ile Gln Lys Pro
1 5 10 15
Tyr Gln Leu Phe His Tyr Tyr Phe Leu Leu Glu Lys Ala Pro Gly
20 25 30
Ser Thr Val Ser Asp Leu Asn Phe Asp Thr Asn Ile Gln Thr Ser
35 40 45
Leu Arg Lys Leu Lys His His His Trp Thr Val Gly Glu Ile Phe
50 55 60
His Tyr Gly Phe Leu Val Ser Ile Leu Phe Phe Val Phe Val Val
65 70 75
Phe Pro Ala Ser Phe Phe Ile Lys Leu Pro Ile Ile Leu Ala Phe
80 85 90
Ala Thr Cys Phe Leu Ile Pro Leu Thr Ser Gln Phe Phe Leu Pro
95 100 105
Ala Leu Pro Val Phe Thr Trp Leu Ala Leu Tyr Phe Thr Cys Ala
110 115 120
Lys Ile Pro Gln Glu Trp Lys Pro Ala Ile Thr Val Lys Val Leu
125 130 135
Pro Ala Met Glu Thr Ile Leu Tyr Gly Asp Asn Leu Ser Asn Val
140 145 150
Leu Ala Thr Ile Thr Thr Gly Val Leu Asp Ile Leu Ala Trp Leu
155 160 165
Pro Tyr Gly Ile Ile His Phe Ser Phe Pro Phe Val Leu Ala Ala
170 175 180
Ile Ile Phe Leu Phe Gly Pro Pro Thr Ala Leu Arg Ser Phe Gly
185 190 195

Phe Ala Phe Gly Tyr Met Asn Leu Leu Gly Val Leu Ile Gln Met

200 205 210

Ala Phe Pro Ala Ala Pro Pro Trp Tyr Lys Asn Leu His Gly Leu

215 220 225

Glu Pro Ala Asn Tyr Ser Met His Gly Ser Pro Gly Gly Leu Gly

230 235 240

Arg Ile Asp Lys Leu Leu Gly Val Asp Met Tyr Thr Thr Gly Phe

245 250 255

Ser Asn Ser Ser Ile Ile Phe Gly Ala Phe Pro Ser Leu His Ser

260 265 270

Gly Cys Cys Ile Met Glu Val Leu Phe Leu Cys Trp Leu Phe Pro

275 280 285

Arg Phe Lys Phe Val Trp Val Thr Tyr Ala Ser Trp Leu Trp Trp

290 295 300

Ser Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Ile Gly

305 310 315

Gly Ala Met Leu Ser Leu Thr Val Phe Glu Phe Thr Lys Tyr Lys

320 325 330

Tyr Leu Pro Lys Asn Lys Glu Gly Leu Phe Cys Arg Trp Ser Tyr

335 340 345

Thr Glu Ile Glu Lys Ile Asp Ile Gln Glu Ile Asp Pro Leu Ser

350 355 360

Tyr Asn Tyr Ile Pro Val Asn Ser Asn Asp Asn Glu Ser Arg Leu

365 370 375

Tyr Thr Arg Val Tyr Gln Glu Pro Gln Val Ser Pro Pro Gln Arg

380 385 390

Ala Glu Thr Pro Glu Ala Phe Glu Met Ser Asn Phe Ser Arg Ser

395 400 405

Arg Gln Ser Ser Lys Thr Gln Val Pro Leu Ser Asn Leu Thr Asn

410 415 420

Asn Asp Gln Val Pro Gly Ile Asn Glu Glu Asp Glu Glu Glu Glu

425 430 435

Gly Asp Glu Ile Ser Ser Ser Thr Pro Ser Val Phe Glu Asp Glu

440 445 450

Pro Gln Gly Ser Thr Tyr Ala Ala Ser Ser Ala Thr Ser Val Asp

455 460 465

Asp Leu Asp Ser Lys Arg Asn

470

[Claims]

[Claim 1] An isolated gene coding for a protein which regulates aureobasidin sensitivity.

[Claim 2] An isolated gene as claimed in Claim 1 which is contained in a DNA fragment represented by a restriction enzyme map as specified in any of Fig. 1 to Fig. 3.

[Claim 3] An isolated gene as claimed in Claim 1 which is hybridizable with a gene of Claim 2.

[Claim 4] A process for cloning a gene of Claim 1 characterized by using a gene of Claim 2 or 3 or a part thereof as a probe.

[Claim 5] A nucleic acid probe which comprises a sequence consisting of 15 or more bases and is hybridizable with a gene of Claim 1.

[Claim 6] An antisense DNA of a gene which codes for a protein regulating aureobasidin sensitivity.

[Claim 7] An antisense RNA of a gene which codes for a protein regulating aureobasidin sensitivity.

[Claim 8] A recombinant plasmid containing a gene of Claim 1.

[Claim 9] A transformant having a recombinant plasmid of Claim 8 introduced thereinto.

[Claim 10] A process for producing a protein regulating aureobasidin sensitivity characterized by culturing a transformant of Claim 9 and collecting the protein regulating aureobasidin sensitivity from the culture.

[Claim 11] An isolated protein regulating aureobasidin sensitivity which is encoded by a gene of Claim 1.

[Claim 12] An antibody against a protein of Claim 11.

[Claim 13] A process for detecting a protein regulating aureobasidin sensitivity which comprises using an antibody of Claim 12.

[Claim 14] A process for detecting a gene coding for a protein regulating aureobasidin sensitivity which comprises the hybridization with the use of a nucleic acid probe of Claim 5.

[Claim 15] A process for screening an antimycotic which comprises using a transformant of Claim 9 or a protein of Claim 11.

[Claim 16] An isolated gene coding for a protein which regulates aureobasidin sensitivity substantially as herein described with reference to any one of the Examples.

[Claim 17] A transformant having an isolated gene coding for a protein which regulates aureobasidin sensitivity substantially as herein described with reference to any one of the Examples.

[Claim 18] A process for producing a protein which regulates aureobasidin sensitivity substantially as herein described with reference to any one of the Examples.

Dated this 16th day of May 1994

<u>TAKARA SHUZO CO., LTD.</u>
By their Patent Attorney
GRIFFITH HACK & CO.

[Designation of Document] Abstract

[Abstract]

[Object] To provide a protein regulating the sensitivity to an antimycotic aureobasidin, a gene coding for this protein, the use thereof, an antibody for the protein and the use thereof.

[Constitution] An isolated gene coding for a protein regulating aureobasidin sensitivity. A process for cloning the gene with the use of the gene or a part of the same as a probe. A nucleic acid probe being hybridizable with the gene. An antisense DNA or RNA of the gene. A recombinant or transformant having the gene contained therein. An isolated protein regulating aureobasidin sensitivity and a process for producing the same by using the transformant. An antibody for the protein. A process for detecting the protein or the gene. A process for screening an antimycotic by using the protein or the transformant.

[Effects] Useful in the diagnosis and treatment for diseases including mycoses.

[Selected Figure] none.

Fig. 1

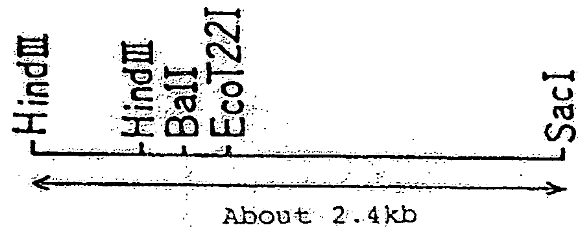


Fig. 2

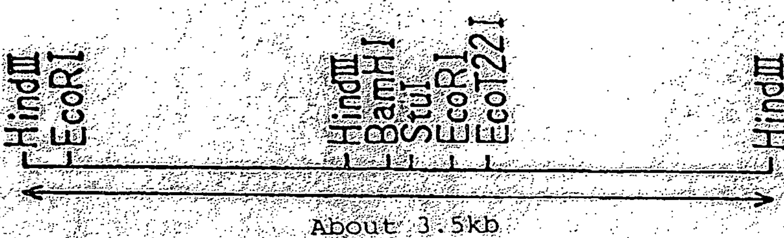


Fig. 3

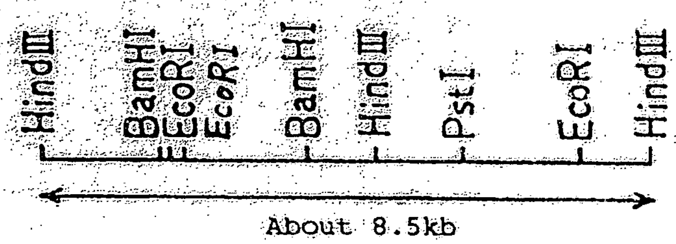
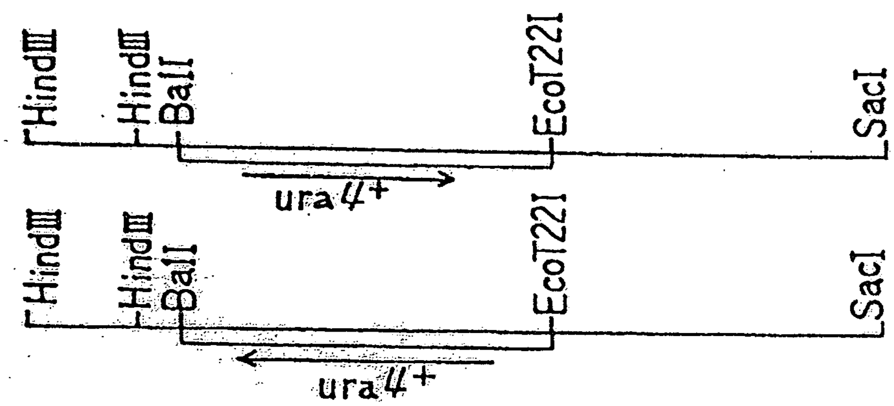


Fig. 4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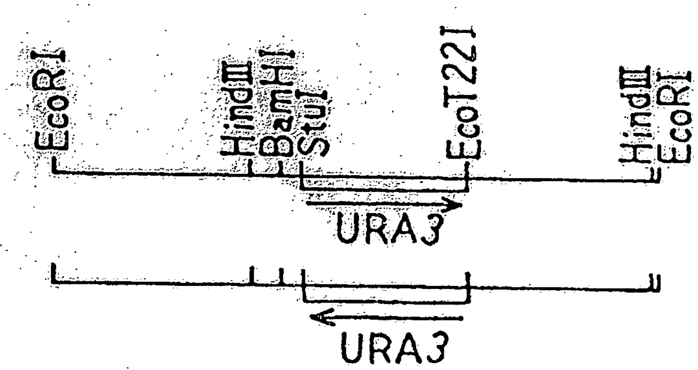
HindIII
BalI
EcoT22I
SacI
ura4+
HindIII
HindIII
BalI
EcoT22I
SacI
ura4+

Fig. 5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BamHI
StuI
EcoT22I
HindIII
EcoRI
URA3
URA3

Fig. 6

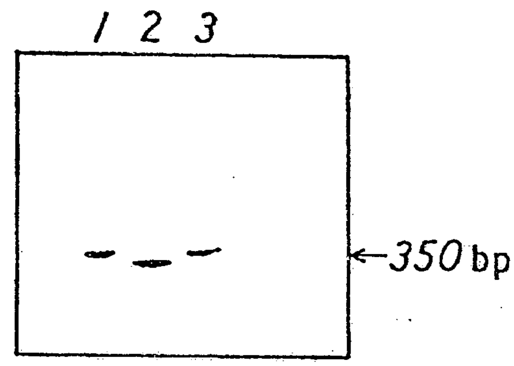


Fig. 7

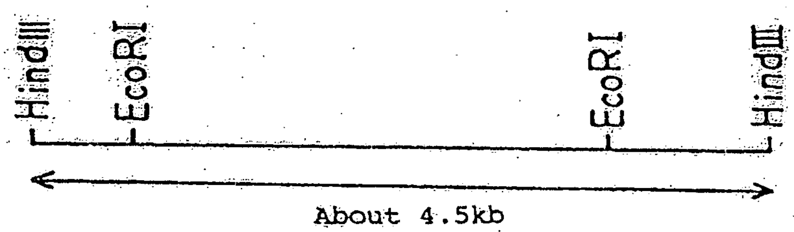
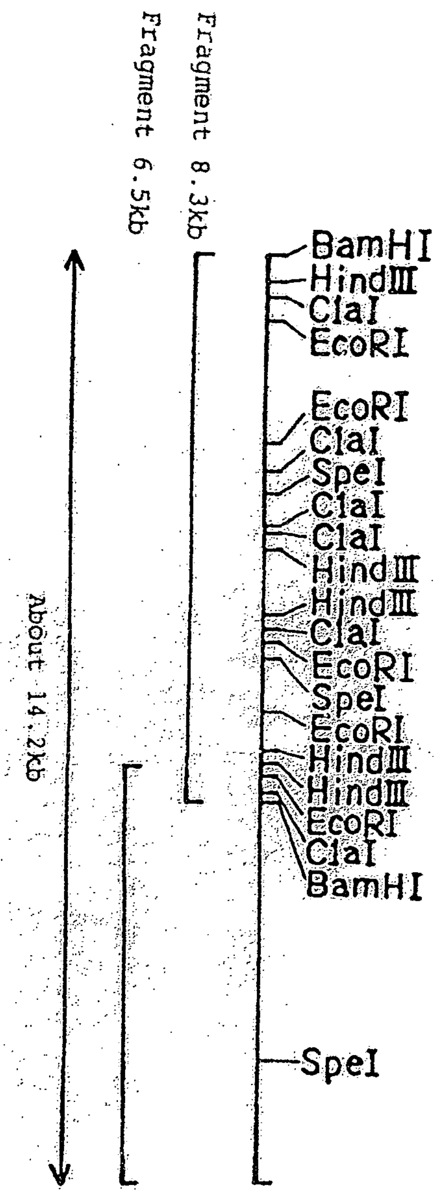


Fig. 8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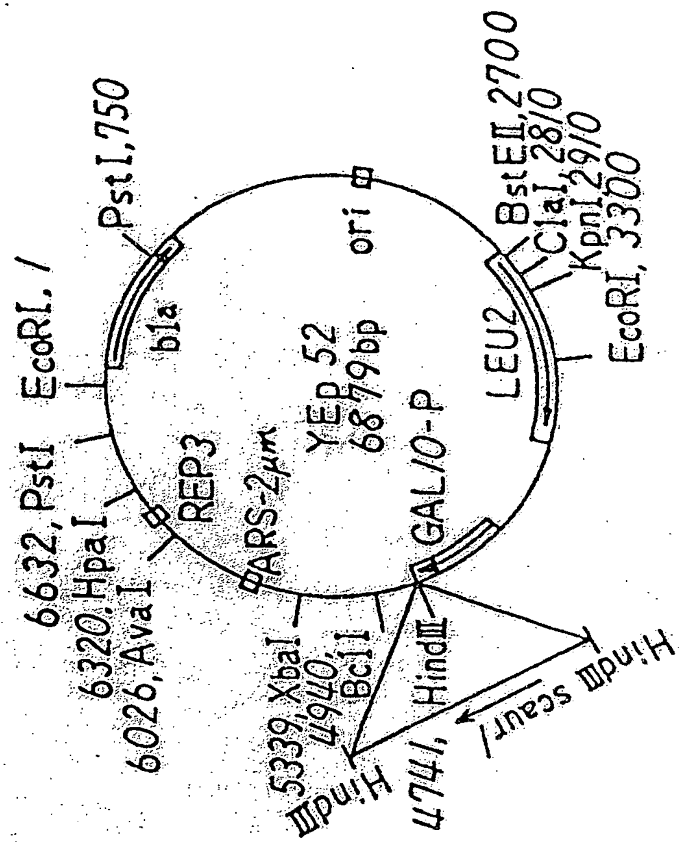
SpeI
EcoRI
HindIII
HindIII
EcoRI
ClaI
BamHI
SpeI

Fig. 9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GAL10-P
LEU2
BstEII,2700
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4940, BclI
HindIII
HindIII
4741, HindIII scar

Fig. 10

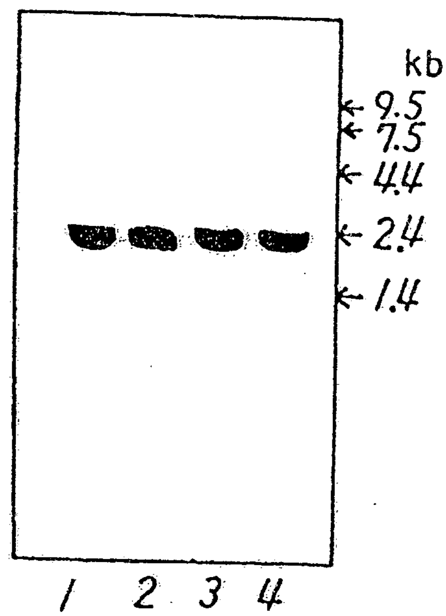


Fig. 11

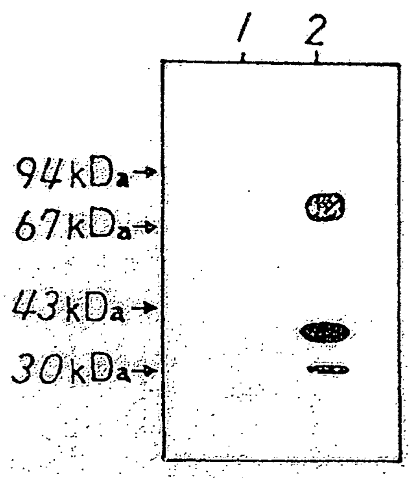
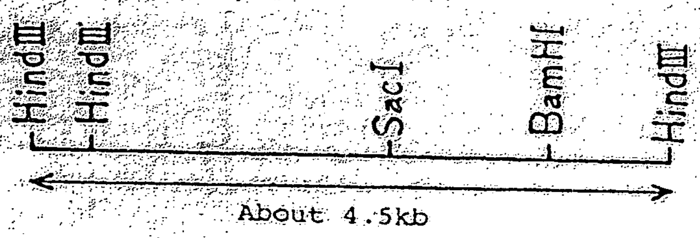

1
2
94kDa
67kDa
43kDa
30kDa

Fig. 12


HindIII
HindIII
SacI
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HindIII
About 4.5kb